Remarks

Amendments to the Claims

Claim 10 has been cancelled as failing to limit the claim from which it depends and lacking proper antecedent basis.

The Claimed Invention

Applicant is the first to recognize that lipoprotein and/or cholesterol levels affects a female's ability to reproduce. Applicant is the first to recognize that SR-BI, by virtue of its role as the only known transporter of cholesterol, which is critical to steroid production, plays a major role in female reproduction by virtue of its role in steroid production, and can therefore be a basis to inhibit pregnancy and to treat disorders characterized by overproduction of steroids.

Applicant demonstrated the criticality of SR-BI, and its role on lipoprotein and cholesterol levels, using SR-BI knockout mice. The homozygous knockout females are unable to carry a fetus to term. Heterozygotes are able to do so. These studies are described in the patent application as filed.

The data presented in the specification clearly demonstrate that multiple compounds have been identified and are representative of widely disparate species, ranging from nucleic acid molecules encoding SR-BI to organic compounds for lowering cholesterol.

In example 5, Applicant demonstrated that transient increases in SR-BI expression following administration of an adenoviral vector encoding SR-BI results in a decrease in cholesterol levels. In example 6, the Applicant demonstrated that SR-BI knockout animals exhibit the opposite phenotype; increased cholesterol levels (see Table 3). Data in example 7

further shows that these animals are also infertile. Antibody blocking studies have also showed similar results using antibodies to block cholesterol transport, resulting in lowered cholesterol levels, as described in Example 8, page 55.

The reagents and methods provided in the present specification were used to subsequently show the restoration of fertility in an SR-BI knockout mouse (or their transplanted oocytes) in the absence of ovarian and/or extraovarian SR-BI expression by manipulations that modify the structure, composition and/or abundance of their abnormal plasma lipoproteins. These manipulations centered around the administration of probucal, a cholesterol lowering drug (Meittinen, *et al., J. Clin. Invest.* 108:1717-1722 (2001)).

The application therefore teaches one skilled in the art that SR-BI is essential for normal female fertility; that decreasing levels of SR-BI activity decreases cholesterol levels and alters lipoprotein levels; and that restoring SR-BI activity normalized cholesterol levels and lipoprotein profiles, with a concurrent increase in steroidogenesis and female fertility. The application further teaches that one can use any number of compounds to alter SR-BI levels: viral vectors to increase SR-BI expression; antibodies to block SR-BI activity and concurrent transport of cholesterol; and organic molecules identified by routine screening assays using SR-BI binding and uptake studies. These compounds alter SR-BI activity either by increasing the amount of transport or by decreasing transport (for example, using viral vectors or antibodies).

The present application, and its analysis of SR-BI knockout mice, ties together fertility and cholesterol level. The direct correlation that exists between cholesterol/HDL and the existence of SR-BI, lies at the core of the claimed method. Many compounds that already exist

for regulating cholesterol levels can be used to inhibit fertility (i.e. pregnancy) via the inhibition of SR-BI expression or activity. The compounds can also be used to treat disorders characterized by elevated steroidal levels. It would not require undue experimentation to identify these known compounds, the patients to be treated, or what constitutes an effective amount. Moreover, one skilled in the art would have no difficulty in identifying the scope of the claimed method in view of the specification, the examples, and the knowledge available to those skilled in the art.

Rejection Under 35 U.S.C. § 112, written description

Claims 1-10, 12, 15, 16 and 20-22 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention.

The Legal Standard for Written Description

"There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed". *Wertheim*, 541 F.2d at 262, 191 USPQ at 96 (CCPA 1976). The written description requirement for a claimed genus may be satisfied through a sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with a known or a disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the Applicant was in possession of the claimed genus (see i)(C), above). See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. On the other hand, there may be a situation where one species adequately supports a genus. See, e.g., *Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326-27.

In the patent context, not all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if, in the knowledge of the art, the disclosed function is sufficiently correlated to a particular, known structure. (*Amgen v. Hoechst Marion Roussell* 314 F.3d 1313 Fed.Cir. 2003).

The Specification Complies with the Written Description Requirement

The claimed invention is based on the clear cut description of the nexus between fertility, steroidal levels and cholesterol levels. The specification is replete with support for this novel connection (see, for example, page 7, lines 21-22; page 13, lines 14-15; page 49, lines 16-20; page 49, lines 21-24 and Example 7). Because of this established connection, the applicant is claiming **all** compounds that alter lipoprotein, LDL, HDL, or cholesterol levels mediated by SR-BI for the purpose of inhibiting pregnancy or decreasing production of steroids in a mammal. The examples in the specification clearly show that, for example, **antibodies** raised against a portion of the extracellular domain of the protein inhibit the selective uptake of HDL and delivery of HDL cholesterol to the steroidogenic pathway in cultured adrenal cells (Example 8, and in particular, Table 4, wherein anti-SR-BI inhibited the production of tritiated steroid derived from tritiated HDL). Adenoviral vectors encoding SR-BI (Example 5) are an additional example

of a compound that has been shown to alter cholesterol levels (as will be discussed below in more detail as it relates to enablement). In each of Examples 3, 5 and 8, the applicant has **reduced to practice a distinct compound** for altering cholesterol levels.

In Example 6, the SR-BI gene was inactivated in embryonic stem cell by standard recombination methods (strategy shown in Example 3). Blastocysts were injected with the embryonic stem cells, producing 24 male chimeric mice. F1 offspring (from crosses between the chimeric mice and wild type females) were either homozygous or heterozygous at the SR-BI locus. F1 intercrosses generated F2 progeny, wherein the males were fertile and the homozygous females (-/- at the SR-BI locus) were unable to produce offspring (Example 7 further discusses the reproductive studies on these mice to make a determination regarding fertility). Example 6 then discusses the resultant elevated levels of plasma cholesterol in which the cholesterol and apolipoprotein profiles of the *heterozygous* mutants were similar to those of wild type controls, except that there was an increase in the amount of cholesterol in the HDL fractions. In the *F2 homozygous* mutant animals (-/- at the SR-BI locus), the cholesterol was found in a large, somewhat heterogeneous peak in the HDL range (*via* FPLC cholesterol analysis). In view of the foregoing results (as discussed in detail in Example 6), one of ordinary skill in the art will readily recognize, not only the direct correlation that exists between cholesterol/HDL and the existence of SR-BI, but also the many compounds that already exist for regulating cholesterol levels. These compounds, well known for altering cholesterol levels, can be used to inhibit pregnancy or decrease steroidal overproduction via the modulation of SR-BI expression or activity. For

further support in this regard, the examiner's attention is again drawn to the attachments to the Amendment filed August 13, 2002.

The legal standard for written description does NOT require that the applicant reduce to practice all of the claimed species that may fall within the claimed genus. In this regard, the Examiner's attention is not only drawn to the three widely disparate types of compounds discussed in the foregoing paragraph, but additionally, and respectfully, drawn to the section in the specification entitled: "I. Inhibitors of SR-BI transport of cholesterol", and the section entitled: "II. Methods of Regulation of SR-BI cholesterol transport to alter steroidogenesis". The description clearly conveys that, in addition to the classes of compounds actually used to show reduction to practice, a number of other molecules were known and could be screened for utility in the claimed method.

The test under 35 U.S.C. 112 was clearly articulated by the Court in *Amgen Inc. v. Hoechst Marion Roussel, Inc. and Transkaryotic Therapies, Inc.* 314 F.3d 1313, 65 USPQ 2d (Fed. Cir. 2003) as being different in the case where, as here, the reagents that could be used in a claimed method were known, and where one was claiming a novel class of compounds. The Court weighed heavily the fact that one was not claiming the class of compounds *per se*, but the use of the compounds. A different degree of description is required where compounds are known and one only needs to provide the criteria for their selection and use - a degree clearly met by applicant.

Rejection Under 35 U.S.C. § 112, enablement

Claims 1-10, 12, 15, 16 and 20-22 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled for inhibiting pregnancy in any animal models other than SR-BI knockout female mice.

The Legal Standard for Enablement

The Court of Appeals for the Federal Circuit (CAFC) has described the legal standard for enablement under § 112, first paragraph, as whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation. See, e.g., *Amgen v. Hoechst Marion Roussell* 314 F.3d 1313 (Fed. Cir. 2003) and *Genentech, Inc. v. Novo Nordisk A/S*, 108 F3d at 165, 42 USPQ2d at 1004 (quoting *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). See also *In re Fisher*, 427 F.2d at 839, 166 USPQ at 24; *United States v. Telectronics, Inc.*, 857 F.2d 778 (Fed. Cir. 1988); and *In re Stephens*, 529 F.2d 1343 (CCPA 1976). The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *M.I.T. v. A.B. Fortia*, 774 F.2d 1104 (Fed. Cir. 1985). In addition, as affirmed by the Court in *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524 (Fed. Cir. 1987), a patent need not teach, and preferably omits, what is well known in the art.

Whether the disclosure is enabling is a legal conclusion based upon several underlying factual inquiries. See *In re Wands*, 858 F.2d 731, 735, 736-737, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir.1988). As set forth in *Wands*, the factors to be considered in determining whether a claimed invention is enabled throughout its scope without undue experimentation include the

quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims. In cases that involve unpredictable factors, "the scope of the enablement obviously varies inversely with the degree of unpredictability of the factors in volved." *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation ' must not be unduly extensive.' *In re Atlas Powder Co., v. E.I. DuPont De Nemours & Co.* , 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir.1984).

As noted in *Ex parte Jackson*, the test is not merely quantitative, since a considerable amount of experiment is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. See *Ex parte Jackson*, 217 USPQ 804, 807 (PTO Bd. App. 1982). The adequacy of a specification's description is not necessarily defeated by the need for some experimentation to determine the properties of a claimed product. *See Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F3d 956, 965-966 63 USPQ2d 1609, 1614 (Fed. Cir. 2002). There is no requirement for examples.

The Specification Complies with the Enablement Requirement

As discussed above, the claims are based on the discovery of *the nexus between fertility, steroidal levels, and cholesterol levels* (see, for example, page 7, lines 21-22; page 13, lines 14-15; page 49, lines 16-20; page 49, lines 21-24 and Example 7). Because of this established connection, the applicant is claiming **all** compounds that alter lipoprotein, LDL, HDL, or cholesterol levels mediated by SR-BI for the purpose of inhibiting pregnancy or decreasing production of steroids in an individual having a steroidal overproduction disorder. One can readily identify patients to be treated. It should be noted that the class of patients does not overlap with the normal class of patients treated with drugs altering cholesterol and/or lipoprotein levels. Most of these individuals are older - in the case of women, cholesterol does not typically increase until after menopause.

The examples have been discussed above. The paper showing restoration of fertility by administration of a cholesterol lowering drug, probucal, also discussed above, provides further support for the claimed method. The mere fact that this data was obtained after the filing date of the application makes the data no less relevant for demonstrating enablement which is asserted in the application, where the paper uses methods and materials known and readily available at the time the application was filed. The role of SR-BI in fertility is clearly established by the examples in the specification, whether it is to be restored in the case of knockout animals with insufficient SR-BI, or inhibited in the case of administering inhibitors of SR-BI. The role of SR-BI in cholesteryl transport was known (see, page 10, lines 19-21).

Because HDL is the only lipoprotein present in substantial amounts in the follicular fluid surrounding the developing oocyte in humans, based on the data in the examples, it is expected that changes in HDL and/or SR-BI in humans may disturb oocyte maturation or function, and thus contribute to infertility. The present application, and its analysis of SR-BI knockout mice, ties together fertility and cholesterol level. The direct correlation that exists between cholesterol/HDL and the existence of SR-BI, lies at the core of the claimed method. Many compounds that already exist for regulating cholesterol levels can be used to inhibit pregnancy, or decrease steroidal production *via*, for example, the modulation of SR-BI expression or activity. It would not require undue experimentation to identify these compounds, the patients to be treated, or what constitutes an effective amount. Moreover, one skilled in the art would have no difficulty in identifying the scope of the claimed method in view of the specification, the examples, and the knowledge available to those skilled in the art.

The following enclosed articles establish that mouse models are accepted models for human gonadotropin signaling pathologies, and thus that the data applicant has provided with respect to the transgenic mice would be expected to be predictive of results in humans.

Burns and Matzuk, "Minireview: Genetic Models for the study of Gonadotropin Actions" Endocrinology 143(8):2823-2835 (2002)

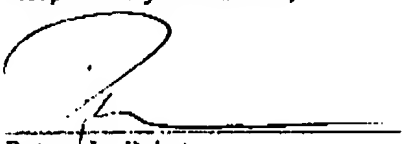
Matzuk and Lomb, "Genetic dissection of mammalian fertility pathways" Nature Cell Biology & Nature Medicine Fertility Supplement (2002) online table

The applicant respectfully asserts that one of skill in the art would understand from the specification which compounds to use, and how to derive appropriate doses with minimal routine

experimentation to practice the claimed method and inhibit fertility or treat a disorder characterized by excessive steroidal production.

For the foregoing reasons, Applicant submits that the claims 1-10, 12, 15, 16, and 19-22 are patentable.

Respectfully submitted,



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Minireview: Genetic Models for the Study of Gonadotropin Actions

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Fertility in both sexes relies on complex physiological and molecular processes with many levels of regulation, and our ability to alter the mammalian genome using transgenic technology has greatly enhanced our understanding of these processes. There are numerous commonalities in human and mouse physiology, and the list of mouse models recapitulating recognized and idiopathic human reproductive defects is growing at an ever-increasing rate. In this review, we focus on genetic models of gonadotropin actions, summarizing features of transgenic mice that phenocopy defects in gonadotropin production and gonadotropin receptor responses seen in patients. In addition, we provide examples of mouse models with genetic alterations influencing pituitary FSH and LH production and their effects. These include: 1) transgenic mice with aberrations in steroid hormone, inhibin, and activin feedback pathways; 2) knockouts that demonstrate specific *in vivo* functions of pituitary transcription factors; and 3) models with alterations in other pituitary hormones, IGF-1, and leptin signaling pathways, which affect both the central and peripheral endocrine axis. What we have to learn from these and other models will continue to revise our conceptions of physiology, identify new targets for contraception, and improve our tools for understanding, diagnosing, and treating cases of human endocrinopathies and pathologies of the reproductive tissues. (*Endocrinology* 143: 2823–2835, 2002)

"Whenever man comes up with a better mousetrap, nature immediately comes up with a better mouse." James Carswell

Mouse Models and Human Gonadotropin Signaling Pathologies

THE HYPOTHALAMIC-PITUITARY-GONADAL (HPG) axis is fundamental to the endocrine control of gametogenesis in mammals, and because of its long-recognized physiological importance, many human mutations disrupting the normal function of the axis have already been characterized, and several relevant mouse models have been engineered. The pituitary gonadotropins, FSH and LH, are central to this endocrine communication. FSH and LH are heterodimeric glycoproteins each comprised of a common α-subunit and a unique β-subunit; functional dimers are synthesized and secreted into the circulation in response to hypothalamic GnRH. The human common α-, FSHβ-, and LHβ-subunits are glycosylated 92-, 111-, and 121-amino acid peptides, respectively (1). FSH and LH elicit intracellular signaling pathways by binding to their respective G protein-coupled transmembrane receptors, FSH receptor (FSHR) and LH receptor (LHR), in somatic gonadal cells to regulate follicular development, ovulation, and steroidogenesis in females, and spermatogenesis, testicular growth, and steroidogenesis in males (2, 3). The human FSHR and LHR proteins are 695- and 701-amino acid glycoproteins, respectively. Loss-of-function and gain-of-function human mutations in components of the HPG axis have been described previously (4–6), and examples are given in Table 1 and illustrated in Fig. 1.

The pulsatile secretion of FSH and LH is regulated by the decapeptide GnRH, also referred to as LHRH. Hypothalamic production of GnRH and signaling through pituitary GnRH receptor (GNRHR; a 328-amino acid G protein-coupled receptor) appear essential to maintaining serum gonadotropins, and ultimately fertility in mammals. Mutations in genes mediating the developmental migration of GnRH-releasing neurons (as seen in the *KAL* gene causing Kallman's syndrome), or aspects of GnRH processing (as seen in mutations in the *PC1* protein processing enzyme) lead to hypogonadotropic hypogonadism (HHG) (7, 8). However, to date, no defined loss-of-function mutations in the GnRH gene itself have been described in patients with HHG (9). Mutations in the GnRHR gene resulting in GnRH resistance have been described in HHG patients who are homozygous or compound heterozygous for missense mutations (6). *In vitro* studies of these mutant GnRHR products indicate that they are hypomorphic mutations that cause deficiencies in GnRH binding or intracellular signal initiation. Clinically, a majority of these patients respond to administration of exogenous GnRH treatment, and in one reported case, pulsatile provision of GnRH was able to induce ovulation and restore a woman's fertility (10).

In contrast to humans, in which no GnRH mutation has been identified, a naturally occurring 33.5-kb deletion truncating the *Gnrh* gene in mice (11) results in a model of he-

Abbreviations: ActRII, Activin receptor type II; CG, chorionic gonadotropin; DAX-1, dosage sensitive sex-reversal-adrenal hypoplasia, congenital critical region on the X chromosome 1, gene 1; Egr, early growth response; ER, estrogen receptor; FSHR, FSH receptor; GHR, GH receptor; GnRHR, GnRH receptor; hCG, human CG; HHG, hypogonadotropic hypogonadism; HPG, hypothalamic pituitary-gonadal; *Inhα*, inhibin α; *lacZ*, β-galactosidase; LHR, LH receptor; MMTV, mouse mammary tumor virus; OTX1, orthodenticle homolog 1; Pit1, pituitary-specific transcription factor 1; POUHF1, POU domain, class 1, transcription factor 1; PRL, prolactin; Prop1, paired like homeodomain factor 1, product of Pit1; Sf-1, steroidogenic factor-1; *Srγ*, sex determining region of chromosome Y; WT-1, Wilms tumor 1.

TABLE 1. Genetic causes of reproductive axis phenotypes in patients

Gene	Clinical presentation (autosomal recessive unless specified)	Reference
GnRH	No ORF mutations or deletions are reported, although deletions at the GnRH locus have been described in patients with IHHG	9
GnRHR		
$Gln^{106}Arg$	Hypomorphic alleles; males present with IHHG, low serum testosterone, delayed puberty and oligospermia; females have primary amenorrhea, low serum estrogens, FSH and LH	6
$Arg^{262}Gln$		138
	No activating mutations described	
α-Subunit	No human mutations reported; predicted to result in embryonic lethality because the α-subunit is also shared with hCG in humans	4
FSHβ		
Frameshift Val^{51}	Loss-of-function mutations; females present with primary amenorrhea and infertility; males with azoospermia, low testosterone and high serum LH	139, 140
$Cys^{51}Gly$		
LHβ		
$Glu^{90}Arg$	Loss of function mutation described in a homozygous, infertile male with low serum testosterone, no Leydig cells, and high levels of immunoreactive LH	28
FSHR		
$Ala^{189}Val$	Loss-of-function mutation; females have a defect in follicular maturation; males have decreased testes volume and oligospermia	25, 141
$Asp^{567}Gly$	Activating mutation described in a hemizygous, hypophysectomized but fertile male	142
LHR		
$Ser^{616}Tyr$	Hypomorphic allele; micropenis	143
$Ala^{593}Pro$	More severe loss-of-function mutation; XY individuals develop female external genitalia, intraabdominal testes with no Leydig cells; XX individuals present with amenorrhea	144, 145
$Asp^{578}Gly$	Activating mutation causing autosomal dominant familial male precocious puberty; no female phenotype	32

reditary hypogonadism (*hpg*), which phenocopies the HHG of patients with defects in GnRH production or responsiveness. Hypogonadism is also a feature of a knockout mouse model harboring a null allele at the glycoprotein hormone common α-subunit locus; besides reproductive defects, the knockout mouse is hypothyroid and displays proportional dwarfism owing to loss of TSH function (12). These and other relevant mouse models with reproductive phenotypes are shown in Table 2. No mutations altering the amino acid sequence of the common α-subunit have been described in humans. It has been hypothesized that a deleterious mutation in the human α-subunit gene (*GLYCA*) would result in embryonic lethality because in humans (and not in mice) the α-subunit is also shared with human chorionic gonadotropin (hCG) (4). Embryo-derived hCG maintains ovarian luteal cells, and is thereby required for pregnancy during the first trimester. Substantiating this, the most highly expressed hCGβ-subunit-encoding genes are highly conserved, with no individuals homozygous for a deletion of the *CGβ* cluster (13).

Recently, a second GnRH peptide (GnRH II) (14) and its receptor (type II GnRHR) (15) have been described in several species, including humans. GnRH I (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) and GnRH II (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2) differ by three amino acids, their affinities for the two described G protein GnRH receptors, and their tissues of expression and potential action. Human GnRH II is expressed in several regions of the brain, including the amygdala, caudate nucleus, hippocampus and thalamus, as well as outside the central nervous system in the kidney, bone marrow, prostate, endometrium, and ovary (14, 16, 17). Type II GnRHR is expressed ubiquitously and is down-regulated in multiple tumor cell lines, which has led to the suggestion that it may be involved in inhibiting cell proliferation and prompting differentiation programs (15); indeed, *in vitro* experiments indicate that GnRH II has antiproliferative effects on ovarian carcinoma cells (18). Functional characterizations of GnRH II and its receptor *in vivo* have not yet been reported, and the roles of GnRH II signaling, if any, in gonadotropin physiology remain unknown.

Loss of FSH signaling causes infertility in women (19), and this condition can be modeled by disrupting either the FSHβ (*Fshb*) or *Fshr* loci in mice. Women who are homozygous or compound heterozygous for inactivating ligand (frameshift/truncation and missense) or receptor (missense) mutations exhibit normal preantral follicle development, but no antral-stage follicles capable of ovulation form. These women typically present clinically as cases of primary amenorrhea and sexual infantilism. Both *Fshb* and *Fshr* knockouts in mice phenocopy the human mutations in this regard, displaying female infertility, uterine hypoplasia, and folliculogenesis blocks before antrum formation (20–22). Although estradiol is present in the serum of these mice, the mRNAs encoding P450 side chain cleavage and P450 aromatase estrogenic enzymes are markedly down-regulated in the ovaries of *Fshb* knockout mice (23). Both *Fshb* and *Fshr* knockout females also exhibit high levels of circulating LH (20–22). In both models, this is a progressive effect, with less LH seen in younger mice than in older mice. It is believed that LH and LH-induced androgen synthesis in the *Fshr* model contribute to the development of ovarian sex-cord stromal tumors, which are seen in 92% of these mice by 12 months of age. In these tumors, there is loss of granulosa cell proliferation control

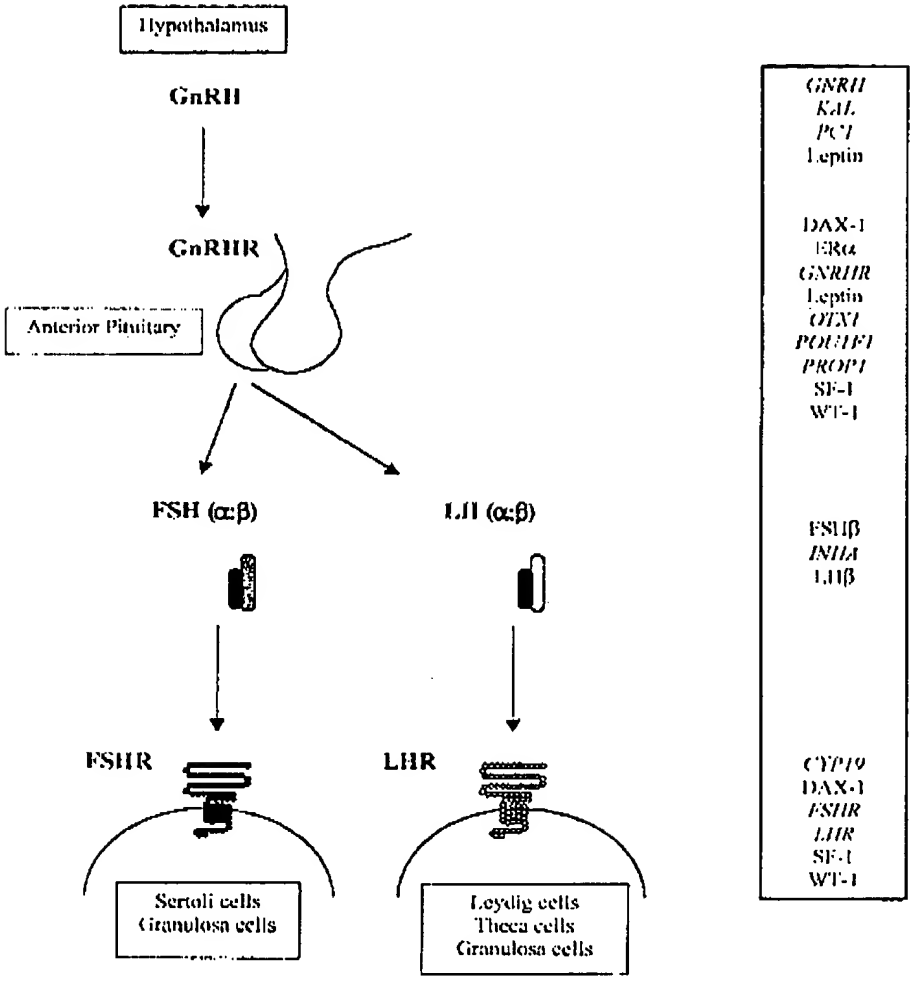

FIG. 1. The HPG endocrine axis is illustrated with several key components of the signaling relay. Within the central portion of the axis, the actions of hypothalamic GnRH mediate the production of the anterior pituitary heterodimeric gonadotropins, FSH and LH. These bind to FSHR and LHR in the periphery to regulate gametogenesis and steroidogenesis. At each level of the axis, mutations in human genes are known to cause defects in the development or function of the reproductive system; examples are listed on the *right*.

programs, accompanied by an up-regulation of Sertoli cell markers, Müllerian-inhibiting substance, and GATA-4 transcription factor (24). In men, FSHβ and FSHR mutations have been associated with variable degrees of impairment of spermatogenesis and sometimes with delayed puberty (4). However, men with *FSHR* null mutations can father children (25). *Fshb* and *Fshr* knockout male mouse models are fertile but have lowered sperm counts and decreased testicular size. Therefore, it seems that FSH activity, while necessary for optimal sexual development and spermatogenesis, is dispensable for male, but not female, fertility in both humans and mice.

A transgenic mouse model overexpressing high levels of human FSH has also been developed (26). The transgenic males demonstrate stimulated Leydig cell function, elevated serum testosterone, and infertility; females exhibit infertility with hemorrhagic and cystic ovaries. These mice may prove valuable in modeling gonadal and more global physiological effects of FSH hyperstimulation. Consistent with the high conservation of FSHβ genes in mice and humans, synthesis of human FSHβ under its own promoter in the gonadotropes of mice lacking the endogenous FSHβ-subunit restores normal fertility in male and female mice (27). Moreover, partial rescue of the knockout phenotype was observed in mice ectopically expressing human FSH (human α- and FSHβ-subunits) under the direction of the mouse metallothionein-1 promoter (27). Together, these studies demonstrate functional conservation between mouse and human FSHβ promoter elements, as well as between the two FSHβ peptides in terms of their abilities to assume glycosylation patterns, dimerize with the mouse α-subunit, route to secretory granules independent of LH, and bind and activate the mouse FSHR.

Loss of isolated LH signaling function can arise from defects in the unique LHβ-subunit or LHR. One loss-of-function missense mutation in the hormone subunit was identified in an infertile man presenting with low testosterone levels, Leydig cell hypoplasia, and elevated immunoreactive but functionally ineffective LH (28). Several LHR mutations have been discovered to cause defects in receptor

TABLE 2. Selected genetic mouse models with reproductive axis phenotypes

A. Models of overexpression and ectopic expression

Construct	Reproductive phenotype	References
mMT promoter and activin/inhibin βA sequence	Males with high expression in testes are infertile; seminiferous tubules show patchy lesions and vacuoles	146
Dax1 overexpresser (Dax regulatory region)	XY mice with a weak *Sry* allele develop as females	90
Bovine glycoprotein hormone-α promoter and diphtheria toxin coding sequence	Pituitary gonadotrophs lost; mice are hypogonadal	84
mMT-1 promoter and human FSH α:β subunits	Infertility in both sexes with hyperstimulation of gonadal steroidogenesis in males and hemorrhagic and cystic ovaries in females	26
mMT I promoter and follistatin (*Fst*) coding sequence	Progressive infertility in both sexes; males have somatic cell and germ cell defects; females have folliculogenesis defects	79
GH overexpressers (multiple constructs studied)	Compromised reproduction in both sexes	147
Cosmid transgene containing the human CGβ cluster	Genes are transcribed in the mouse placenta, cerebral cortex, pituitary, and adrenal glands	148
Liver promoter and leptin coding sequence (*skinny* mice)	Females have accelerated puberty and late-onset hypothalamic hypogonadism	149
mMT I promoter and rat inhibin α coding sequence	Female subfertility; low serum levels of FSH and LH	64
Mifepristone inducible transgene overexpressing inhibin A from liver	Females have a block in folliculogenesis at the early antral stage; males have decreased testis size	150
Bovine glycoprotein α promoter and bLHβ-hCG fusion	Female infertility, polycystic ovaries, granulosa/theca cell tumors	33
P450 aromatase (*Cyp19*) overexpression (human ubiquitin C and MMTV promoters)	Cryptorchidism and Leydig hyperplasia or tumor development in males	54, 56
Human GnRH promoter and SV40 T antigen sequence	GnRH neurons do not migrate appropriately; mice are hypogonadal	151
Inhibin α subunit promoter and SV40 T antigen sequence	Gonadal tumors and progressive decreases in gonadotropins	152

B. Knockout models and naturally occurring loss-of-function mutations

Gene	Reproductive phenotype (in homozygotes unless specified)	References
Activin receptor-type IIA (*Acvr2*)	Infertility in females and delayed fertility in males; small gonads	76
Activin/inhibin βB subunit (*Inhbb*)	Females are subfertile and litters variably do not survive postnatally	73
Dax1 (DSS-AHC region on the human X)	Males are infertile with progressive degeneration of the germinal epithelium	153
Egr1 (NGFI-A transcription factor)	Female infertility; LH deficiency	94, 95
Early growth response 4 (*Egr4*)	Infertility in males; most germ cells undergo apoptosis during pachytene stage	99
Estrogen receptor α (ERα) (*Esr1*)	Females are infertile with hemorrhagic ovarian cysts and uterine defects and high circulating LH; males develop disruptions of the seminiferous epithelium	40, 47
Estrogen receptor β (ERβ) (*Esr2*)	Females are subfertile; males are fertile, but develop prostate hyperplasia	154
FSH β-subunit (*Fshb*)	Female infertility; preantral block in folliculogenesis; males fertile with decreased testis size	20
FSH receptor (*Fshr*)	Female infertility; block in folliculogenesis prior to antral formation	21
γ-Glutamyl transpeptidase (*Ggtp*)	Both males and females are hypogonadal and infertile; phenotype corrected by feeding mice *N*-acetylcysteine	123
Glycoprotein hormone α-subunit	Males and females are infertile; hypogonadal due to FSH and LH deficiency	12
GnRH (*Gnrh*)	*hpg* mice of both sexes show hypogonadotropic hypogonadism and infertility	11
GH receptor (*Ghr*)	Females show delayed puberty and prolonged pregnancy; males have low FSH and testosterone	114, 155
Inhibin α (*Inha*)	Female infertility; male secondary infertility; granulosa/Sertoli tumors	60
IGF 1 (*Igf1*)	Females are hypogonadal and infertile; impaired antral follicle formation; males are infertile with low testosterone	119
Large tumor suppressor (*Lats1*)	Knockout mice develop tumors; pituitary hyperplasia with decreased LH, PRL, and GH levels	156
Leptin (*Lep*)	*ob/ob* mice are obese and infertile with hypogonadotropic hypogonadism in both sexes	124, 125
Leptin receptor (*Lepr*)	*db/db* are obese and infertile with hypogonadotropic hypogonadism in both sexes	126
LH Receptor (*Lhcgr*)	Underdeveloped sex organs and infertility in both males and females; spermatogenesis arrested at round spermatid stage; folliculogenesis block prior to antral stage	29, 157
Neuronal Helix-Loop-Helix 2 (*Nhlh2*)	Males are infertile and hypogonadal; females are fertile when reared with males	158
Neuronal insulin receptor	Mice exhibit hypothalamic hypogonadism; impaired spermatogenesis and follicle maturation	159

TABLE 2. *Continued*

Gene	Reproductive phenotype (in homozygotes unless specified)	References
Otx	Prepubescent dwarfism and hypogonadism; progressive recovery of spermatogenesis, follicular development, and fertility	93
P450 aromatase (*Cyp19*)	Females are infertile; ovaries do not form CL; mice progressively develop hypergonadotropism and cystic ovaries; males develop progressive infertility with defects in spermatogenesis	50–52
Pit1	*Snell* dwarf mice have multiple anterior pituitary hormone deficiencies and hypogonadism	160
Prolactin (*Prl*)	Females are infertile with irregular estrus cycles	111
Prolactin receptor (*Prlr*)	Males infertile; females infertile related to compromised ovulation, fertilization and preimplantation development	112
Prop1	*Ames* dwarf mice have multiple anterior pituitary hormone deficiencies and hypogonadism	161
Steroidogenic factor-1 (SF-1) (*Nr5a1*)	Gonadal and adrenal agenesis in both sexes	80
TSH (*Tshb*)	*hyt* mice are hypothyroid; females show continuous diestrus, and poor response to gonadotropin-induced superovulation	109
Vitamin D receptor (*Vdr*)	Defects in estrogen biosynthesis in males and females; elevated serum gonadotropins	58
Wilms tumor homolog (*Wt1*)	Embryonic lethality with gonadal agenesis	162

activity of variable severity. Phenotypes range from micropenis and hypospadias in the case of hypomorphic alleles to male pseudohermaphroditism and female infertility associated with a barrier to preovulatory follicle development, ovulation, and luteinization (4). These conditions are partially modeled by targeted deletion of the *Lhr* locus in mice; homozygotes demonstrate normal prenatal sexual development but are infertile (29). Male *Lhr* knockouts have defects in testicular growth and descent, Leydig cell hypoplasia, and a block in spermatogenesis at the round spermatid stage. Female knockouts have underdeveloped ovaries and uteri, and their ovaries do not contain preovulatory stage follicles or corpora lutea; thus, the *Lhr* knockout in female mice very closely models the pathology of women with LH resistance. It is notable that *hpg* (GnRH I mutant) male mice closely phenocopy the *Lhr* mutant male mice, whereas the *hpg* female phenotype mimics that of *Fshb* and *Fshr* knockouts at early timepoints (~6 wk of age) but reflects a combined FSH/LH signaling defect at later points. This observation is consistent with studies showing that androgen administration to *hpg* male mice can restore spermatogenesis (30). Interestingly, the androgens produced by Leydig cells in response to LH may not be as crucial to spermatogenesis as estrogens that are then synthesized from androgen substrates. Provision of exogenous estradiol to *hpg* males increases testis weight and rescues qualitatively normal spermatogenesis in the absence of measurable androgens (31).

Gain-of-function mutations of LHR have been described in families with autosomal dominant male precocious puberty (32). Although no mouse models phenocopy this disorder, transgenics have been engineered to overexpress the bovine LHβ-subunit with a C-terminal extension of the hCGβ-subunit (bLHβ-CTP); there is a prolonged serum half-life of the chimeric LH hormone (33). These *in vivo* studies corroborate findings of Boime and colleagues (34, 35), who demonstrate that the presence of the hCGβ C terminus of several of the glycoprotein hormone β-subunits (*e.g.* hCGβ, FSHβ) extends the circulating half-life. Interestingly, female bLHβ-CTP transgenics have a 10-fold increase in circulating immunoreactive LH and exhibit impaired ovulation, a prolonged luteal phase, ovarian cysts, and granulosa/thecal cell tumors on some genetic backgrounds (36). In addition, in immature transgenic females, enhanced LH and steroid hormones cause precocious follicular development and vaginal opening (37). Studies of these transgenic mice may shed light on the roles of LH in polycystic ovarian syndrome and the development of postmenopausal ovarian stromal tumors, as well as identify genetic modifiers of these phenotypes (36).

Mouse Models of Aberrant Steroid Hormone Feedback to the Pituitary

Gonadotropins promote peripheral steroid production by inducing the expression of gonadal steroidogenic enzymes; steroid hormones then produced feedback to negatively regulate pituitary FSH and LH production (1, 38, 39). This paradigm is supported by studies of mouse models with alterations in steroid hormone receptors or steroid biosynthesis that develop secondary anomalies in gonadotropin production. For example, knockout mice lacking an estrogen receptor, ERα, which is normally expressed in the hypothalamus, pituitary and gonads, exhibit female infertility associated with anovulation and the development of hemorrhagic, polycystic ovaries by 20–22 d of age (40, 41). The disruption to estrogen feedback signaling results in overexpression of gonadotropin subunit mRNAs in the pituitary (42) and chronically elevated serum LH (43). High levels of circulating LH are key to the ovarian pathogenesis in the ERα knockout mice, and treatment with a GnRH antagonist precludes ovarian cyst formation (44). In contrast, female mice lacking ERβ, which has a relatively restricted pattern of expression, are fertile, and preliminary studies indicate that there is no change in serum LH (45). ERβ signaling plays some role, however, in the regulation of the hypothalamic-pituitary-gonadal endocrine axis, at least in the absence of ERα activity. Double knockout females lacking ERα and ERβ have higher serum LH levels that do ERα single knockouts, and exhibit a different ovarian phenotype in which granulosa cells take on a Sertoli cell-like morphology and express Sertoli cell markers, Müllerian-inhibiting substance, sulfated glycoprotein-2, and *Sox9* (46).

Male ERα knockout mice are infertile because of a dis-

ruption of fluid reabsorption from the lumen of the epididymis, which is important for concentration and maturation of spermatozoa; increased pressure from the accumulation of fluid leads to testicular atrophy and degeneration of the seminiferous epithelium in mature males (47). This phenotype does not have an obvious relationship to the only clinical case of an ER mutation, that of a man with estrogen resistance caused by a homozygous nonsense mutation in ERα (48). The 28-yr-old patient presented with incomplete epiphyseal closure and continued linear growth into adulthood. Biochemical analyses provided evidence of increased bone turnover, and bone mineral density was low. Serum estradiol, FSH, and LH levels were elevated; serum testosterone was normal. Interestingly, the patient also had impaired glucose tolerance and hyperinsulinemia, and ERα knockout male mice have increases in total body fat and in serum cholesterol and leptin levels after sexual maturity (49). These findings underscore the importance of estrogens in the bone and lipid metabolism, and have important implications for the use of hormonal contraceptions and estrogen replacement therapies in women, and appreciating the effects of exposure to environmental estrogens.

Estrogen production is abrogated in the P450 aromatase knockout mouse model, the female reproductive phenotype of which closely resembles that of ERα knockout mice. Female P450 aromatase knockouts have high serum levels of LH and FSH, are infertile owing to a block in follicular development and ovulation defects, and develop hemorrhagic ovarian cysts by 21–23 wk of age (50, 51). Male P450 aromatase knockouts develop progressive infertility characterized by an arrest in early spermiogenesis, germ cell apoptosis, high circulating LH, and Leydig cell hyperplasia (52). Heritable aromatase deficiency has been described in patients with mutations at the aromatase locus (*CYP19*) (53). In the absence of fetal aromatase (and therefore placental estrogens), there is an elevation of testosterone and androstenedione in both maternal and fetal circulations. This causes pseudohermaphroditism in homozygote females, and virilization of the mother during her pregnancy. In the absence of ovarian aromatase activity, young girls exhibit hypergonadotropic hypogonadism and develop follicular cysts; with the onset of puberty, the ovaries become enlarged and further polycystic and there is progressive virilization. In males, the aromatase deficiency is associated with macroorchidism, high circulating FSH, LH, and testosterone, as well as hyperinsulinemia and abnormal plasma lipids. In both women and men, aromatase deficiency is associated with delayed epiphyseal fusion and osteopenia (53).

An aromatase-overexpressing mouse model has been developed to study the effects of enhanced conversion of androgens to estrogens on male reproduction. These mice carry a transgene expressing human aromatase under the constitutive human ubiquitin C promoter, which is active in multiple mouse tissues by embryonic d 15. These mice have cryptochidism with Leydig cell hyperplasia and disrupted spermatogenesis, as well as underdevelopment and defects of accessory sex organs. Serum hormone assays reveal elevated estrogen, reduced testosterone, and reduced FSH, with no change in average LH levels but a reduction in LH level variation (54). The cryptorchidism is likely due to a disruption of steroid effects during prenatal development. The etiology of the Leydig cell hyperplasia is less clear, though both cryptorchidism and exposure to estrogens have been associated with testicular tumorigenesis in mice, and cryptorchidism is a risk factor in humans (55). Leydig cell tumorigenesis is reported in a second aromatase-overexpressing transgenic model in which the aromatase coding sequence is expressed under the mouse mammary tumor virus (MMTV) promoter. The expression leads to Leydig cell tumors that express high levels of ERα, and up-regulation of a G1→S phase cell cycle promoter, cyclin D1, known to be modulated by estrogen exposure (56). The MMTV promoter in this mouse model also drives aromatase expression in the mammary glands and this results in preneoplastic lesions (57). Interestingly, vitamin D has also been recently shown to be important for estrogen biosynthesis in both the ovary and testis. Studies of knockout mice lacking the vitamin D receptor reveal attenuation of gonadal P450 aromatase activity, histological abnormalities of the uterus, ovary, and testis consistent with estrogen deficiency, and elevated levels of LH and FSH (58). Together, the phenotypes of these models indicate important *in vivo* roles for the sex steroid hormones as mediators of the HPG endocrine axis and also as cell cycle controllers and differentiation factors in hormonally responsive peripheral tissues in both males and females.

Peptide Hormone Feedback to the Pituitary

In addition to steroid hormones, peptide endocrine factors released from the gonads affect pituitary FSH and LH production. Inhibins (α:βA and α:βB heterodimers) and activins (βA:βA and βB:βB homodimers, and βA:βB heterodimers) are members of the TGFβ superfamily named for their respective abilities to suppress and enhance FSH production. These peptides are synthesized in granulosa cells of the ovary and Sertoli cells of the testis and are also found in other tissues where they have been implicated in diverse biological processes (59). Male and female knockout mice lacking the α-subunit (*Inhα*$^{-/-}$), and therefore depleted of the biological effects of both inhibins, have high circulating activins and FSH, and develop steroidogenic sex-cord stromal tumors of the granulosa cell and Sertoli cell lineage. These tumors are associated with a cachexia-like wasting syndrome, which typically causes death between 8 and 18 wk of age (60, 61). In addition, superovulation experiments in younger knockout females indicate that there are defects in late stages of follicle development, and transplant experiments demonstrate important paracrine roles of ovarian inhibins in maintaining the granulosa cell phenotype and averting the formation of Sertoli tubule-like structures (62). FSH and LH are critical to the processes of tumorigenesis in these mice, as double mutant mice homozygous for the hypogonadal (*hpg*) mutation at the *Gnrh* locus and the *Inhα* knockout allele do not develop tumors (63), and *Fshb* and *Inhα* double knockouts develop tumors with later onset and a less aggressive course, a finding that is particularly pronounced in double knockout males (26). In contrast to the *Inhα*$^{-/-}$ model, mice that overexpress the rat inhibin α coding sequence under the control of the metallothionein promoter have reduced FSH levels in both sexes and elevated LH levels in females. In addition,

females exhibit subfertility owing to a decrease in the number of ovulated oocytes, a defect that can be corrected with the provision of exogenous gonadotropins (64).

Recent clinical evidence indicates that inhibins may regulate human gonadotropin production, and ultimately affect the duration of a woman's reproductive potential. A point mutation in the human *INHa* gene, resulting in a nonconservative amino acid change ($Ala^{257}Thr$), has been associated with premature ovarian failure (65). The mutation was found in three patients who presented with secondary amenorrhea, low serum estrogens, and elevated gonadotropins, at ages 16, 20, and 24 yr of age. The authors of the study suggest that a decrease in the bioactivity of the mutant inhibin led to persistently enhanced gonadotropins, and premature depletion of ovarian follicular reserves. Low levels of circulating inhibins and high levels of FSH have been associated with premature ovarian failure before, though this study is the first to implicate inhibins in the condition's etiology. We anticipate that future studies of inhibin mutations, and clinical assessments of inhibin and FSH levels preceding ovarian failure, will be informative.

When inhibin α knockout mice are castrated, they develop steroidogenic tumors of the adrenal cortex, indicating that inhibin signaling is key to proliferation control in the adrenal glands, as well as the gonads (61). Gonadal tumors and the development of adrenal cortical tumors upon castration are also features of a transgenic model expressing the SV40 T antigen (Tag) under the control of the mouse inhibin α promoter (66, 67). Despite similarities in their phenotypes, the endocrine environments in which gonadal tumors develop in these two mouse models are quite distinct, with a progressive decrease in serum LH and FSH in the Tag overexpresser being noted as ovarian tumorigenesis proceeds (68). Nevertheless, there is a critical function of the gonadotropin hormones in this model, as is demonstrated by the findings that: 1) the *lpg* mutation prevents the Tag mice from developing both gonadal and adrenal tumors (69); 2) adrenal tumor cells express LHR and respond to LH by up-regulating steroid production *in vitro* (70); and 3) suppression of serum LH by exogenous testosterone administration precludes tumor development (69). Similarly, elevated serum LH in the bLHβ-CTP transgenic model promotes LHR expression and induces steroidogenesis in the adrenal cortex (71). Such LH action may also be involved in tumorigenesis in the inhibin α knockout models, where there is a striking induction of LHR and P450 aromatase mRNAs in adrenal tumors (our unpublished data). Together, these mouse models provide evidence for important regulatory roles of inhibins and LH on nongonadal steroidogenesis and tumorigenesis.

Homozygote mice with a null mutation engineered at the activin/inhibin βA locus (*Inhba*$^{-/-}$) die neonatally due to craniofacial defects that prevent suckling, and until more recently this had precluded further studies pertaining to the role of activin subunit in reproduction. The null phenotype, however, can be rescued by replacing the activin/inhibin βA coding sequence with that of the activin/inhibin βB gene, conferring the activin/inhibin βA expression pattern on this related sequence (63% amino acid identity). Knock-in mice demonstrate enlarged external genitalia, hypogonadism, and diminished female fertility, indicating unique and previously unrecognized functions of the activin/inhibin βA protein product in reproduction (72). Serum FSH levels in these mice are slightly increased, though whether this reflects loss of pituitary inhibin α:βA signaling, enhanced activin βB:βB signaling, or is simply secondary to gonadal defects remains to be investigated. Knockout mice lacking a functional βB locus (*Inhbb*$^{-/-}$) have developmental defects in eyelid closure, prolonged gestation, and a failure of mothers to nurse their litters (62, 73). The latter is also a primary characteristic of oxytocin knockout mice (74), and therefore, together these phenotypes substantiate that activin βB is an important in the induction of this hypothalamic/posterior pituitary hormone (75).

Signaling pathways that mediate activin and inhibin effects are complex, and no receptor or downstream signaling protein mutations have been described that phenocopy the mutant models lacking functional ligands. In the case of activin signaling, however, transgenic mouse models with altered expression of activin-interacting proteins have elucidated specific aspects of activin function in FSH regulation. Activin receptor type II (ActRII) has been shown to relay activin-mediated induction of FSH, as knockout mice lacking ActRII have suppressed FSH levels in the pituitary and serum; LH levels are not affected. Mutant ActRII mice also exhibit gonadal pathologies at least in part due to the lack of FSH, including a delay in fertility and reduced testicular growth in males, and infertility, underdeveloped uteri, follicular atresia and reduced numbers of corpora lutea in females (76). Bioactivities of activin dimers are modulated by their association with a binding protein, follistatin; the interaction is believed to antagonize activin functions in the pituitary (62, 77). Follistatin knockout mice have numerous embryonic defects and die in the perinatal period (78), but the role of follistatin in controlling FSH levels can be appreciated from studies of transgenics overexpressing follistatin under the control of the metallothionein promoter. Lines of mice with widespread expression of the transgene exhibit suppression of serum FSH, and defects in gonadal growth and gametogenesis that can be partially ascribed to FSH deficiency (79).

Transcriptional Control of the Gonadotropin Genes

Knockout mouse models have led to the functional characterization *in vivo* of several pituitary transcription factors important in mediating gonadotropin expression or providing for the survival of neuroendocrine cell populations in the hypothalamus and pituitary. One of these factors is steroidogenic factor-1 (SF-1), originally identified because of its role in directing the expression of cytochrome P450 steroid hydroxylases in the ovary, testis and adrenal gland. Knockout mice lacking SF-1 have profound defects in endocrine development that affect multiple levels of the HPG axis. Newborn SF-1 knockout mice have gonadal and adrenal agenesis, female sex traits, absence of the ventromedial hypothalamic nucleus, and impaired gonadotrope expression of GnRHR, FSH, and LH (80–82). Heterozygote mice are phenotypically normal. To circumvent the complexity of the SF-1 null phenotype and establish its role specifically in pituitary hormone production, tissue-specific SF-1 knockout

mice have been engineered (83). In these mice, a portion of the endogenous SF-1 locus is marked for excision by Cre-recombinase enzyme by the introduction of tandem *loxP* sites, and a Cre-recombinase-encoding transgene is expressed under the α-glycoprotein hormone subunit promoter specifically in cells of the anterior pituitary. In both males and females, the pituitary SF-1 loss-of-function caused sexual infantilism and hypogonadism, and gonadotrope cells failed to express appreciable levels of GnRHR, FSH, or LH (83). Essentially, all gonadotropic effects of the anterior pituitary are lost in these mice, the hypogonadism being as pronounced as when gonadotrope cells are ablated by targeted toxin expression (84), but there is no defect in gonadal or adrenal development during embryogenesis. In several respects, the SF-1 null mouse phenocopies the effects of a heterozygous mutation in the human *FTZF1* gene encoding SF-1. The mutation has been described in a single patient (85) and is a loss-of-function 2-bp missense change affecting the DNA binding domain of the SF-1 protein and eliminating its recognition of the SF-1 canonical nucleotide sequence. The XY female patient presented with adrenal failure in the first 2 wk of life and had streak gonads with poorly differentiated tubule structures. Despite similarities to the knockout model, there was elevation of gonadotropin hormones in this patient in response to administration of GnRH. It is possible that FSH and LH production during this test relied upon the function of SF-1 transcribed from the normal allele.

The reason for the SF-1 haploinsufficiency seen in humans and not mice remains unclear, though dosage sensitivity is also a hallmark of two other human conditions involving related transcriptional regulators and their roles in gonadal development and sex determination. WT-1 (Wilms tumor-1) and DAX-1 (dosage-sensitive sex-reversal-adrenal hypoplasia, congenital critical region on the X chromosome 1, gene 1) proteins interact directly with SF-1 to promote and repress, respectively, its transactivation of target genes, including Müllerian-inhibiting substance (86). Heterozygote XY children with a dominant negative mutation of WT-1 have Denys Drash syndrome, characterized by male pseudohermaphroditism, urogenital aberrations, and nephroblastoma (87). In contrast, XY sex reversal can result from duplication of the DAX-1 locus in the dosage-sensitive sex reversal region of the X chromosome (88). Aspects of the Denys-Drash syndrome phenotype are recapitulated in heterozygous mice with a truncation in WT-1 (89), whereas DAX-1 overexpressing transgenics create a dosage-sensitive sex reversal-like condition in mice with a hypomorpic *Sry* (sex determining region of chromosome Y) allele (90). These and other mouse models with sex determination phenotypes are reviewed by Whitworth and Behringer (91).

At birth and throughout adult life, a homeobox-containing transcription factor, OTX1 (orthodenticle homolog 1), is expressed in the pituitary and is involved in the transactivation of several glycoprotein hormone subunit genes. In addition to neurological abnormalities, *Otx*$^{-/-}$ mice exhibit a prepubescent period of dwarfism and hypogonadism owing to decreases in GH, FSH, and LH; the condition corrects itself by 4 months of age and knockout mice then have restored growth and gonadal function (92, 93). The defect in these mice does not include alterations in the hypothalamic expression of GnRH or the pituitary expression of GnRHR. During the hypogonadotropic, hypogonadal period, knockout males had a block in spermatogenesis and females had ovaries devoid of antral follicles and corpora lutea. These histological findings were not seen in older fertile knockouts, in which there was recovery of all stages of sperm and follicle development (93). The *Otx* knockout phenotype is particularly intriguing because of the window in which it is apparent, this being the first example of a mouse model for investigating causes and effects of delayed growth and puberty, and the regulation of temporal-restricted molecular mechanisms in the onset of sexual maturity.

Mutant mouse models have defined *in vivo* functions of the early growth response (Egr) family of zinc finger transcriptional activators in regulating pituitary hormone production, hindbrain development, peripheral nerve myelination, muscle spindle morphogenesis, and spermatogenesis (94–99). Two of these transcription factors are critical in the establishment of the HPG endocrine axis, EGR1 (also known as nerve growth factor 1A) and EGR4. Though the *Egr1* gene is expressed widely during development, the phenotypes of *Egr1* mutant mice are relatively restricted. The first *Egr1* mutant mouse model was engineered by inserting a neomycin selection cassette into the DNA-binding region of the EGR1 coding sequence (*Egr1*^{neo}), and the primary defect is female sterility due to LH insufficiency (94). Homozygote mutant females show loss of estrous cyclicity, uterine hypoplasia, and no luteinized cells in their ovaries, though corpora lutea are evident upon pregnant mare's serum gonadotropin and hCG treatment. FSH is produced in these mice and is up-regulated upon ovariectomy, but LHβ-subunit mRNA expression is critically compromised. By contrast, *Egr1*^{neo} homozygote mutant male mice have no defects in fertility or spermatogenesis (94), though knockout males lacking EGR4 are infertile because of germ cell maturation defects (99). To examine functional redundancies between EGR1 and ERG4, *Egr1*^{neo}, *Egr4*$^{-/-}$ double mutant mice were bred. Interestingly, double mutant males, unlike *Egr1*^{neo} or *Egr4*$^{-/-}$ single mutants, demonstrated low levels of serum LH, low serum testosterone, and atrophy of androgen-responsive organs (100). Because steroidogenesis was restored by the addition of an LH receptor agonist, the defect in these mice appears to be in pituitary production of this gonadotropin (100). Therefore, though EGR1 is critical for LH production in the maintenance of female fertility, in *Egr1*^{neo} mutant males EGR4 compensates by establishing adequate LH to support androgen production. A second *Egr1* mutant mouse line has been generated by targeted insertion of the *lacZ* (β-galactosidase) gene into the *Egr1* locus (*Egr1*^{lacZ}), and these mice have several notable phenotypic differences compared with *Egr1*^{neo} mice (95). In these mice, somatotrope development and production of GH in both sexes is impaired, male fertility is critically compromised (but can be rescued by LH administration), and female infertility cannot be rescued by LH administration and is associated with a down-regulation of LHR. These findings suggest singular and previously unexpected roles for EGR1 and its target genes in nongonadotrope pituitary cells, LH production in males, and LHR production in females. Whether the *Egr*^{neo} allele is a hypomorphic allele or whether the *Egr*^{lacZ} allele

creates aberrations apart from abrogating EGR1 expression remains to be explored.

Other Pituitary Hormones, IGF-1, and Leptin Affect Multiple Levels of the Endocrine Axis

The physiological complexities of the HPG axis can be further appreciated by studies of mouse models with disruptions of endocrine and paracrine factors known to affect central and peripheral components of the axis. Nongonadotrope anterior pituitary cell lineages and their product hormones may play important roles in establishing bidirectional gonadotrope-gonad communications. *Ames* and *Snell* dwarf mice [with mutations in *Prop1* (paired like homeodomain factor 1; prophet of Pit1) and *Pit1* (pituitary specific transcription factor 1) genes, respectively, encoding pituitary transcription factors] have TSH, prolactin (PRL), and GH deficiencies due to defects in thyrotrope, lactotrope, and somatotrope transcriptional regulation. In addition, both mouse models also display HHG, despite the absence of a direct role for the disrupted transcription factors in gonadotrope ontogeny or gene regulation (101–103). Interestingly, HHG and failure to respond to exogenous GnRH is a feature of patients with homozygous or compound heterozygous *PROP1* mutations (with considerable variability depending upon the exact nature of the mutation), but not in patients with even complete loss-of-function mutations of the human PIT1 gene, *POU1F1* (POU domain, class 1, transcription factor 1) (104).

To gain an understanding of the direct and indirect effects of nongonadotropin pituitary hormones on ovarian function represents an important challenge today for endocrinologists and researchers. Thyroid hormone has been implicated in the function of the lactotrope and somatotrope cell lineages in the anterior pituitary, which produce PRL and GH, respectively (105). Clinically, both hypothyroidism and hyperthyroidism in women have been associated with menstrual abnormalities, infertility, and complications in pregnancy (106–108). Moreover, analyses of a genetic mouse model of hypothyroidism (*hyt*) caused by TSH deficiency have demonstrated that thyroid hormone is important for peripheral gonadotropin hormone response and female fertility in mature animals (109). Postpartum hyperprolactinemia suppresses the HPG axis by inhibiting GnRH production (110), and defects in PRL regulation have been associated with reproductive abnormalities in patients, though the roles of PRL signaling outside of mammary tissue are still not well understood. Female knockout mouse models lacking PRL or PRL receptor are infertile due to defects in luteinization, and PRL receptor knockouts also exhibit irregularities in estrous cyclicity, as well as a failure to support oviductal embryogenesis (111, 112).

GH is crucial for growth and the onset of sexual maturity, both by its direct effects binding the GH receptor (GHR) and by the effects of downstream IGF-1. Knockout mice lacking the GHR have growth and reproductive defects. Females exhibit subfertility, and a delay in the onset of sexual maturity that can be corrected by administration of IGF-1 (113). GHR knockout males have low levels of circulating FSH and IGF-1, low plasma testosterone, and an attenuation in their steroidogenic response to exogenous LH owing to down-regulation of LHR in the testes (114). In contrast, transgenic males that overexpress a human GH transgene under the metallothionein promoter have enhanced transcription of FSH and LH mRNAs, increased serum levels of LH, and an increase in LH release in response to GnRH (103). Female transgenics in which bovine GH expressed by the phosphoenol pyruvate carboxykinase promoter, though unable to maintain pregnancies due to PRL deficiencies, have an increase in the numbers of preovulatory follicles and corpora lutea, and a decreased granulosa cell apoptosis in developing follicles (115, 116). This latter phenotype may in part reflect enhanced IGF-1 function in developing follicles (117). There is no readily evident clinical correlate for these phenotypes, and reports of reproductive defects in patients with alterations in GH regulation most commonly describe cases of hypogonadism in patients with acromegaly. GH overexpression in such patients has been associated with amenorrhea in women and testosterone deficiency in men, even in the absence of hyperprolactinemia (118).

Many biological effects of GH have been attributed to its induction of IGF-1 in peripheral tissues, and studies of *Igf1* knockouts have revealed important functions of this growth factor in prenatal and postnatal growth, as well as within the gonads. *Igf1* knockout males and females are infertile. In males, there is a marked reduction of plasma testosterone, and an inhibition of LH-mediated testosterone production in testicular organ culture experiments (119). In females, there are hypoplastic uteri and no ovulatory response to exogenous gonadotropins (119). Further examination of the *Igf1* null ovaries revealed a block in follicular development reminiscent of that of the FSH knockout mouse and a lack of FSHR expression; this finding and the coexpression of *Igf1* and *Fshr* mRNAs in healthy, growing follicles has led to the proposal that IGF-1 up-regulates FSHR and is needed for FSH induction of steroidogenesis (120). Consistent with a role for IGF-1 in steroidogenesis, both girls and boys with Laron syndrome (primary GH resistance) treated with IGF-1 develop elevated serum androgen levels and secondary effects of androgens (121, 122). Therefore, it seems that GH may influence gonadal functioning both by promoting central FSH and LH production, and by enabling gonadotropin response in the gonads, directly and through up-regulation of IGF-1. It should be noted that other physiological parameters influence IGF-1 levels and activities; metabolic defects in γ-glutamyl transpeptidase mice lead to decreases in IGF-1 levels and similar reproductive phenotypes (123).

Just as GH has pleiotropic effects on the HPG axis, leptin, a protein released from adipocytes, acts upon endocrine circuits at multiple locations. Leptin-deficient *ob/ob* mice are obese, infertile, and have characteristics of HHG with enhanced functioning of negative feedback pathways on gonadotropin production reminiscent of immature animals (124, 125). Similarly, leptin receptor-deficient *db/db* mice are obese and hypogonadal (126). *In vitro* studies have indicated that leptin normally functions at both the level of the hypothalamus (enhancing GnRH production), and at the level of the anterior pituitary (enhancing FSH and LH production) to promote the establishment of adult circulating gonadotropin levels (127). Interestingly, leptin may function dichoto-

mously in HPG axis functioning, up-regulating GnRH, FSH, and LH production centrally, but down-regulating steroidogenesis in peripheral tissues (128–131). To understand the effects of leptin and body fat content on the reproductive endocrine axis in humans represents a field of research with important implications for health care practices. Lean persons have decreased circulating leptin levels compared with obese persons (132). It may be speculated that low leptin levels contribute to the suppressed GnRH, low gonadotropins, abnormal glycosylation of gonadotropins, and delay of menarche or amenorrhea observed in young women athletes and anorexics (133, 134). Obese patients have elevated serum leptin levels but may exhibit leptin resistance so that some biological effects of leptin are attenuated. Roles that leptin signaling and other metabolic regulation pathways play in the development of the altered sex steroid profile and amenorrhea seen in obese women remain to be elucidated. Human mutations resulting in obesity have been described affecting both leptin (135, 136) and the leptin receptor (137). In the case of the leptin receptor mutation, two sisters homozygous for a nonsense mutation presented with early-onset obesity, attenuated levels of growth hormone and IGF-I, hypothalamic hypothyroidism, and did not undergo pubertal development. Their endocrine profile showed low levels of estrogens and gonadotropins that persisted without response to GnRH administration. This clinical case underscores the importance of metabolic pathways in the control of the reproductive axis and other endocrine communications, and the continued utility of the *ob/ob* and *db/db* mouse models to study leptin pathologies in humans.

Conclusions

Cases of human disease and transgenic mouse models offer powerful means for appreciating the molecular components of endocrine pathways, including the direct, indirect and compensatory results *in vivo* of their aberrant functioning. Loss-of-function mutations produce hypomorphic and null alleles in mice that recreate deficiency and resistance syndromes in humans. Transgenic overexpressers and targeted subtle mutations may mimic activating mutations or the effects of hypersecretion syndromes in patients. In some instances, mutant mice closely phenocopy human disorders, stand as important proofs-of-principle, and provide us with models for testing our understanding of etiologies and potential medicinal interventions. In some, unexpected phenotypes call our attentions to unrecognized endocrine relationships, and the pathophysiological bases for previously inexplicable clinical observations. These genetic models will continue to reveal to us aspects of the human endocrine system.

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review

Genetic dissection of mammalian fertility pathways

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The world's population is increasing at an alarming rate and is projected to reach nine billion by 2050. Despite this, 15% of couples world-wide remain childless because of infertility. Few genetic causes of infertility have been identified in humans; nevertheless, genetic aetiologies are thought to underlie many cases of idiopathic infertility. Mouse models with reproductive defects as a major phenotype are being rapidly created and discovered and now total over 200. These models are helping to define mechanisms of reproductive function, as well as identify potential new contraceptive targets and genes involved in the pathophysiology of reproductive disorders. With this new information, men and women will continue to be confronted with difficult decisions on whether or not to use state-of-the-art technology and hormonal treatments to propagate their germline, despite the risks of transmitting mutant genes to their offspring.

Despite advances in assisted reproductive technologies, infertility is a major health problem worldwide. Approximately 15% of couples are unable to conceive within one year of unprotected intercourse. The fertility potential of a couple is dependent on the coordinated and combined functions of both male and female reproductive systems. Anatomic defects, gametogenesis dysfunction, endocrinopathies, immunologic problems, ejaculatory failure and environmental exposures are significant causes of infertility. Although several infertility disorders are associated with defined genetic syndromes (for example, cystic fibrosis and Turner's Syndrome[1,2]), almost a quarter of clinical infertility cases of either sex are idiopathic in nature, in part as a result of a poor understanding of the basic mechanisms regulating fertility. It is thought that genetic defects underlie many of these unrecognized pathologies. On the basis of over 200 infertile or subfertile genetic mouse models (see Supplementary Information Table; also see ref. 3), it is not surprising that the diagnosis of idiopathic infertility is common in the clinic[4,5].

In this review, we discuss causes of mammalian infertility with an emphasis on the genetic basis of fertility defects in humans and mice. Animal models have defined key signalling pathways and proteins involved in reproductive physiology[6]. Mouse models have been produced by spontaneous mutations, fortuitous transgene integration, retroviral infection of embryonic stem cells, ethylnitrosurea (ENU) mutagenesis and gene targeting technologies[7,8]. These mutations affect all aspects of reproduction, including ovarian development and function, testis determination, spermatogenesis, sperm function, genital tract development and function, sexual behaviour, fertilization and early embryonic development, and therefore have contributed much to our understanding of infertility. For example, male infertility is observed in the spontaneous mutant models hypogonadal (*hpg*)[9] and testicular feminization (*tfm*)[10] and in models created by transgene integration, such as the *kisimo* mouse model (which arose by transgene disruption of the *Tieg* gene[11]) and retroviral disruption of the *Bclw*[12], *Mtap*[13] and *Spnr*[14] genes. These models are improving our knowledge of the genetic basis of mammalian infertility and suggest that in the future, clinical technologies must advance to enable analysis of many more genes when an infertile couple enters the clinic. Currently, karyotype analysis, sequence analysis of the cystic fibrosis transmembrane conductance regulator gene and Y chromosome deletion analysis (for males) are the only genetic tests commonly offered to infertile patients[4,5].

Where it all begins

Reproductive development and physiology are evolutionarily conserved processes across eutherian mammalian species and many other vertebrates, including marsupials[15], amphibians, reptiles, birds and fish[16–19]. Several genes required for vertebrate fertility are also highly conserved in evolution, with orthologues in *Drosophila melanogaster* (for example, *vasa* (DDX4), *fat facets* (DFFRY) and *boule* (DAZ)[20–22]). Propagation of the vertebrate germline requires development of the gonads, the site of future gamete production. The indifferent gonad forms during foetal development, primordial germ cells enter the gonad primordium and the tissue eventually differentiates along a female (ovarian) or male (testicular) pathway; this differentiation dictates the formation of the secondary sex organs[19]. Although there may be spatiotemporal variations of these processes in different species (for example, in mice and humans, gonadal sex determination occurs *in utero*, whereas in marsupial mammals, it occurs after birth), they eventually yield ovaries that produce eggs or testes that generate spermatozoa.

Defects in sexual differentiation pathways can cause infertility in mice and humans of both sexes (Fig. 1)[23–25]. In 1959, through the analysis of human XO (Turner syndrome) females and XXY

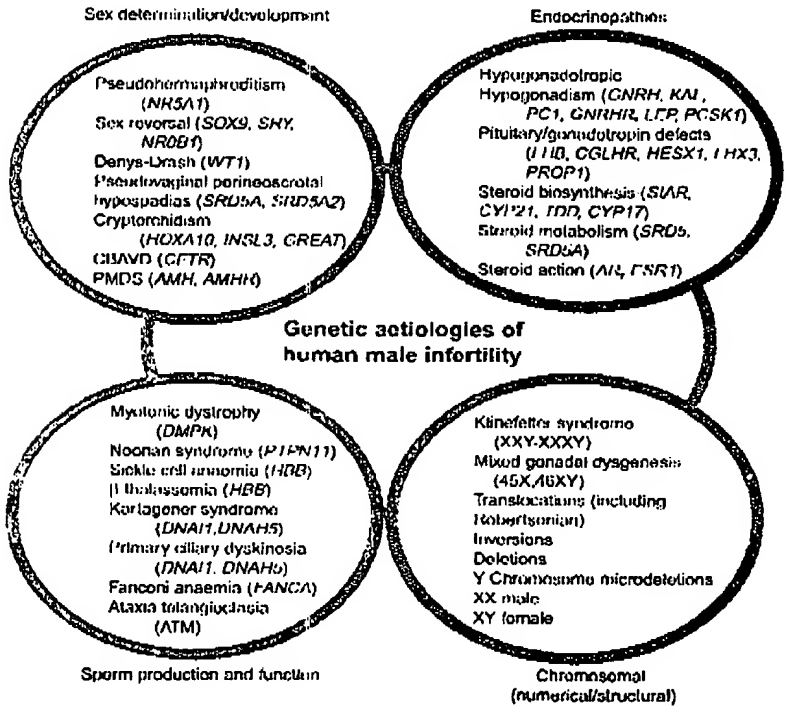

Figure 1 **Genetic aetiologies of human male infertility.** Developmental disorders causing human male infertility result from a failure of gonadal development or testis determination, endocrinopathies, well known genetic syndromes, and numerical and structural chromosomal abnormalities (translocations, deletions and inversions).

(Kleinfelter syndrome) males, as well as XO and XX female mice and XY male mice, it was concluded that the Y chromosome was male determining[26–28]. Subsequent chromosomal and genetic studies of humans and mice with sex reversal syndromes and infertility revealed that many XX males have translocations of a small piece of the Y chromosome, so that the sex determining region Y gene (*SRY*) results in testis development. Similarly, XY females often have inactivating mutations in the *SRY* gene, resulting in the development of ovaries[29,30]. A critical role of *Sry* in sex determination was confirmed by showing that the expression of an *Sry* transgene in an XX mouse causes testis formation, and physical and behavioural sex reversal[31]. Most *SRY* mutations disrupt the high mobility group (HMG) box of the SRY protein; not surprisingly, this region is highly conserved among different species[32]. HMG box-containing proteins typically bind and significantly bend DNA and function as transcription factors or facilitators of transcription. Several genes upstream and downstream of SRY in the sex determination pathway are now known (reviewed in ref. 23). For example, XY female sex reversal correlates with a duplication of the human X-linked gene *DAX1* (ref. 33) or haploinsufficiency of the autosomal *SOX9* gene[32,34–36]. Interestingly, whereas an extra Y chromosome (that is, XYY) has little effect on human male fertility because of the selected loss of the extra Y during spermatogenesis[37], Klinefelter (XXY XXXXY) males account for 10–15% of azoospermic patients[38].

From a distance, they will come

In both sexes, the primordial germ cells (PGCs) are defined histologically as alkaline-phosphatase-positive embryonic cells[39,40]. In the mouse, these cells divide rapidly under the influence of transforming growth factor-β (TGF-β) superfamily signals; knockout models lacking bone morphogenetic protein-4 (BMP-4) or BMP-8b, or the downstream cytoplasmic-to-nuclear relay proteins, SMAD1 and SMAD5, have defects in PGC development[41–44]. At mid-gestation, the PGCs begin one of the longest journeys of any mammalian cell, migrating from their origin at the base of the yolk sac, along the hind-gut, to eventually enter the genital ridge. Factors required for this migration in humans are unknown, although chemoattractants and cell adhesion factors have been implicated[45]. In the mouse, mutations in Kit receptor (KITR) and Kit ligand (KITL) genes block PGC migration, causing infertility, but not altering sexual differentiation[46].

Few known human mutations result in a reduction of the PGC or follicle pool, although girls with Turner's syndrome (partial or complete X-chromosome monosomy), have streak (remnant) gonads with no oocytes. Many Turner's syndrome cases with ovarian failure seem to be caused by loss of the short arm of the X chromosome[2]. Among the candidate Turner's syndrome ovarian failure genes are *ZFX*, *BMP15*, *UBE1* and *USP9X* (ref. 2). An absence of *Zfx* in mice results in a loss of germ cells and subfertility in both sexes[47]; loss of *BMP15* in sheep causes a block at the primary follicle stage and infertility[48]. Studies in XO mice suggest that abnormal chromosomal segregation contributes to germ cell problems[49], indicating that multiple factors are responsible for these human ovarian abnormalities.

Death in the germline

In females, quiescent primordial follicles (a non-growing oocyte surrounded by squamous granulosa cells) form during prenatal life in humans and post-natally in mice. Recruitment of these follicles during

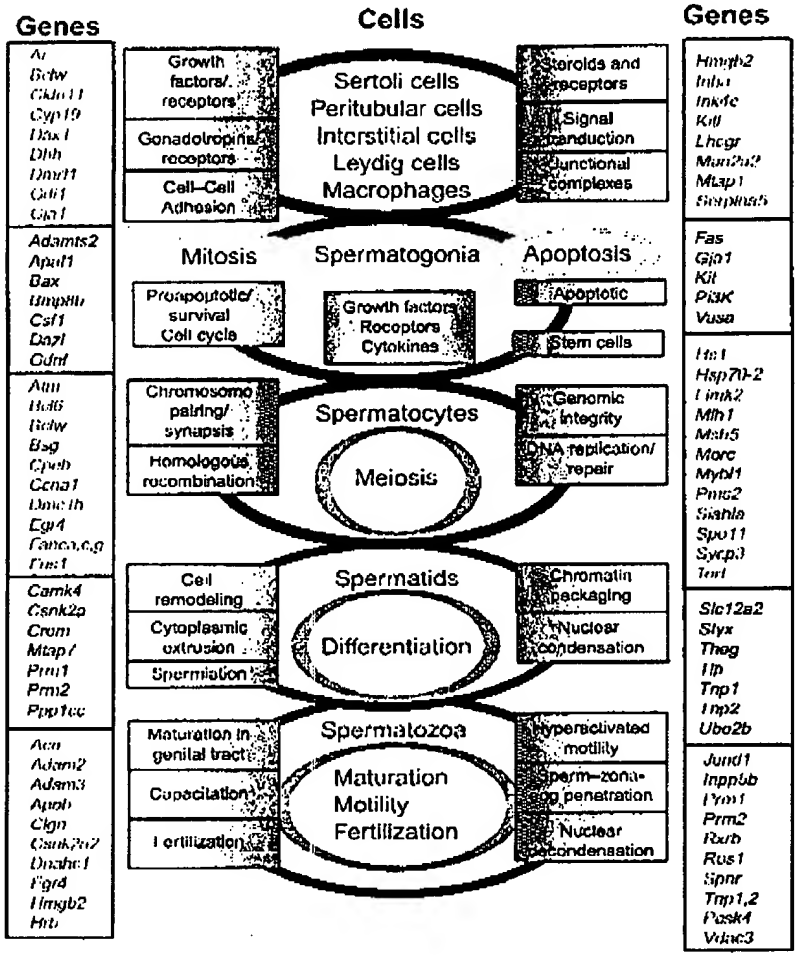

Figure 2 **Genes involved in the regulation of male reproduction in the mouse.** Spermatogenesis requires a complex interaction of the various cellular compartments of the testis (seminiferous epithelium containing spermatogenic cells, Sertoli cells and peritubular myoid cells, the interstitial cell compartment containing the steroidogenic Leydig cells, macrophages, and other interstitial cells, and the vasculature). Targeted mutation of the genes shown affects specific testicular cell types and reproductive function, resulting in male infertility or subfertility in the mouse (detailed in the Supplementary Information table).

ovarian folliculogenesis permits further growth and development of oocytes. In males, spermatogenesis is characterized by three specific functional phases: proliferation, meiosis and spermiogenesis. The proliferative phase in the testis begins early in embryonic development, and with the exception of a brief period when spermatogonia arrest during late foetal and early postnatal life, they proliferate actively throughout life. Spermatogonial stem cells were one of the first recognized examples of adult stem cells capable of rejuvenating spermatogenesis after toxic insult[36,51]. In contrast, the formation of primordial follicles in females defines a finite endowment of oocytes. Between the time of ovary development and reproductive sequence, there is a precipitous drop in the number of oocytes. In humans, seven million foetal germ cells at 20 weeks are reduced to two million oocytes at birth, and eventually to 300,000 at puberty[52,53]. Thus, factors that prenatally and postnatally regulate germ cell survival in the ovary can prolong the reproductive lifespan.

The spermatogonia proliferation rate, the highest in the body, is well regulated; thus, it is not surprising that genes involved in growth (for example, *Kit*, *Csf* and *Bmp8b*) and apoptosis are also required for normal spermatogonia (Fig. 2). This stage of spermatogenesis is also noteworthy for its inefficiency; in rats, 75% of spermatogonia do not survive to become mature sperm[54]. A balance of anti-apoptotic members of the BCL2 family (that is, BCL2, BCL6, BCLX, and BCLW) and the pro-apoptotic BAX protein is extremely important in the regulation of germ cell survival prenatally and postnatally in both sexes, and in response to toxins in the ovary[55–57]. It is possible that defects in this delicate balance of cell proliferation and cell death contribute to the clinical pathology of hypospermatogenesis (all cellular elements of the testis are present, but at low cellularity). In the mouse, an absence of BCLX results in a complete loss of germ cells before birth in both males and females; furthermore, a lack of BCLW results in a partial reduction of PGCs in females, whereas an absence of BCL2 results in only decreased oocyte numbers postnatally[12,58,59]. Absence of either BAX or BCLW causes male infertility, and absence of BCL6 causes male subfertility, again suggesting that a balance of apoptotic/anti apoptotic factors is necessary for normal spermatogenesis. In contrast, a prolonged female reproductive lifespan occurs in the absence of BAX in mice, consistent with its pro-apoptotic role[56]. Polycyclic aromatic hydrocarbons in cigarette smoke and air pollution bind to the aryl hydrocarbon receptor to stimulate transcriptional activation of *Bax*, thereby enhancing apoptosis and oocyte loss[60]. In the future, factors that inhibit the BAX pathways or stimulate the anti-apoptotic pathways could prolong the reproductive lifespans of women.

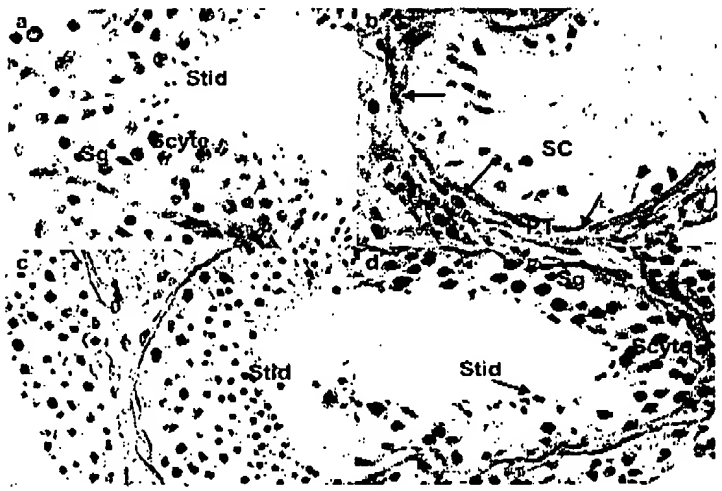

Figure 3 **Spermatogenic failure in the human.** Spermatogenesis in the human is characterized by six stages that are present in a mosaic fashion in the seminiferous tubule. **a**, Normal human spermatogenesis with Sertoli cells (SC) spermatogonia (Sg) towards the basal portion of the tubule, spermatocytes (Scyte) and maturing spermatids (Stid) located towards the lumen of the tubule. The tubules are surrounded by peritubular myoid cells (PT). The interstitial area contains the steroidogenic Leydig cells that secrete testosterone. **b**, An example of the most severe testicular pathology, with a total absence of germ cells and a Sertoli cell only pathology. Mild thickening of the peritubular layer is also observed (arrows: peritubular fibrosis). **c**, A late maturation arrest. The most mature cell type present is the round spermatid. **d**, An example of hypospermatogenesis, where all cell types are present in the testis, but with a low level of cellularity within the seminiferous tubule.

Meiosis, recombination, the integrity of the genome and more death

Meiosis is a process of cell division that is unique to germ cells and is required for the production of healthy haploid gametes (reviewed in greater detail in ref. 62). This process is evolutionarily important for both the integrity and diversity of species, as recombination of homologous chromosomes occurs during prophase of the first meiotic division, helping to orient chromosomes on the meiotic spindle, as well as introducing genetic variability. Male spermatogenesis is initiated postnatally (in mice at postnatal day 7) and is a continuous process producing spermatozoa. Proliferating spermatogonia differentiate and enter meiosis as spermatocytes. In contrast, oogenesis is initiated prenatally (in mice at embryonic day 13), arrests initially at the diplotene stage of meiotic prophase, resumes with the preovulatory luteinizing hormone (LH) surge and arrests again after the first polar body is released before fertilization.

Despite sexual dimorphism in meiosis, many regulators of the process are common to the germ cells of both sexes. In the absence of these proteins, prophase arrest and accompanying germ cell death occur in male and female germ cells. Infertility in both sexes is observed in knockout mice lacking the recombination and DNA damage/mismatch repair proteins, SPO11, DMC1, ataxia telangiectasia (ATM), MSH4, MLH1, and MSH5 (refs 63–74). Mutations in *ATM* and Fanconi anaemia (*FA*) complementation-group-protein genes result in fertility defects in humans and mice of both sexes (Figs 1,2). ATM is involved in DNA metabolism and cell cycle checkpoint control[75], whereas FA is a hereditary chromosomal instability syndrome[76]. FA men are hypogonadal, oligospermic and rarely fertile; FA women can experience premature ovarian failure in their 20s. Several FA mouse models have been created and display reduced fertility[77]. Thus, similar mechanisms for germline monitoring are conserved in mammals and in both sexes.

When the germline 'proofreading' system goes awry in an otherwise 'normal' individual, there are major consequences. Despite a normal somatic karyotype, sperm collected from oligospermic men exhibit an increased frequency of chromosomal abnormalities[78,79]. Aneuploidy is the most common genetic abnormality in humans[80], and the common trisomies (for example, trisomy 21 (Down's syndrome) and trisomy 18)) arise primarily in the children of ageing women through non-disjunction defects during the first meiotic division[81]. These findings are exemplified in mice lacking synaptonemal complex protein 3 (SYCP3), which functions in synapsis (pairing) of the homologous chromosomes during meiosis. *Sycp3* knockout males are infertile; females are subfertile, exhibiting loss of aneuploid embryos[82,83]. Interestingly, germline deletions resulting in Duchenne muscular dystrophy (DMD) more often arise during oogenesis, whereas DMD point mutations result more commonly from spermatogenic failure[84]. This suggests that some proofreading mechanisms during male and female gametogenesis may differ (see also ref. 80).

Hormones take control

After sexual maturity, all stages of spermatogenesis (male) and folliculogenesis (female) are observed, the end result in each case being gamete production. Hypothalamic pituitary control of gonadal somatic cells is critical for fertility in all mammals and in both sexes (Fig. 3; reviewed in refs 85–88). Gonadotropin releasing hormone (GnRH) from the hypothalamus regulates the pituitary gonadotrope production of follicle stimulating hormone (FSH) and LH, α β heterodimers that share a common α subunit with placental human chorionic gonadotropin (hCG) and pituitary thyroid stimulating hormone (TSH). Spontaneous deletion of the hypothalamic GnRH encoding sequences in mice (that is, *hpg*),

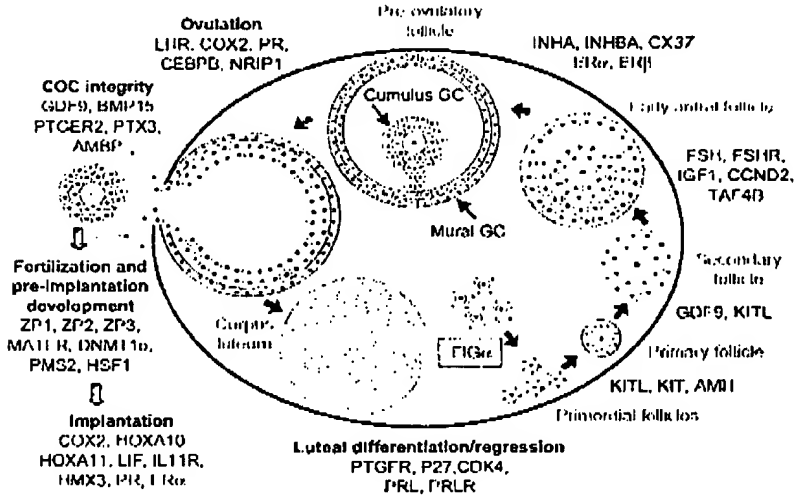

Figure 4 **Female fertility proteins.** Knockout mouse models have defined key proteins that function at various stages of follicle formation, folliculogenesis, ovulation, and post-ovulatory events. FIGα is required for primordial follicle formation, and several proteins are needed for oocyte and granulosa cell (GC) growth and differentiation, ovulation, and the integrity of the cumulus oocyte complex (COC) (reviewed in the Supplementary information table).

mutation of the human GnRH processing enzyme gene (*PC1*), disruption of developmental migration of the GnRH neurons in human Kallman Syndrome, or mutation of the GnRH receptor gene (expressed in gonadotropes), results in hypogonadotropic hypogonadism (HHG) and infertility (Fig. 1). Loss-of-function mutations in the pituitary expressed FSHβ genes and gonadal-expressed FSH receptor genes decrease testis size and spermatozoa counts in men and male mice, and cause a block in folliculogenesis and infertility in women and female mice. This emphasizes the conservation and importance of these signalling pathways. Similarly, pituitary gland development and downstream steroidogenic pathways are conserved in humans and mice and are critical for fertility in both sexes. For example, a homozygous *Prop1* missense mutation causes multiple pituitary defects in the *Ames* dwarf mouse, including defects in gonadotrope differentiation and infertility in all female and most male mice. Similarly, human *PROP1* mutations cause combined pituitary hormone deficiency, including HHG and infertility (Fig. 1).

Members of the steroid receptor superfamily and their transcriptional coactivators (for example, AR, ER, PR, RXRβ, SF1, DAX1, and SRC1) are pivotal in the regulation of reproductive function. Disruption of any of the genes involved in androgen biosynthesis, metabolism and action negatively impact male development, spermatogenesis and function. Spontaneous mutations of the X-linked androgen receptor gene in XY mice (that is, *tfm* (*testicular feminization*)) and humans result in individuals with abnormal testes, no ductal system and external female genitalia[89,90] (http://ww2.mcgill.ca/androgendb). Absence of steroid 5α reductase, which converts testosterone to dihydrotestosterone, results in external female genitalia, prostate absence in XY humans and developmental disruption of the male ductal system (that is, seminal vesicles and prostate)[90,91]. Similarly, mutations in the orphan nuclear receptors, steroidogenic factor-1 (SF1) and DAX1, have been described in mice and humans; mutations in *DAX1* cause almost universal HHG in adult humans (Fig. 1).

Not surprisingly, oestrogen and progesterone are key to early folliculogenesis and corpus luteum maintenance of early pregnancy in the female[92]. Targeted deletion of oestrogen receptor α in mice revealed that it is also required for male fertility and for male and female sexual behaviour[93]. Similarly, the progesterone receptor (also required for female fertility) is important in sexual behaviour in the mouse[94]. In the evaluation of the infertile couple, assessment of circulating hormone levels (FSH, LH, testosterone, prolactin and free testosterone in the male; FSH, LH, oestradiol and progesterone levels in the female) can provide important information concerning the function of the hypothalamic–pituitary–gonadal axis and the presence of endocrinopathies.

Spermatogenesis has many unique players

Spermatogenesis requires not only the appropriate hormonal milieu, but also autocrine, paracrine and juxtacrine signalling between the various testicular compartments. The testis is composed of an interstitial cell compartment with androgen producing Leydig cells, and the seminiferous tubule containing Sertoli cells, peritubular myoid cells and germ cells. Whereas follicles are recruited each cycle to enter the ovulatory pathway in females, all stages of spermatogenesis are present at any one time in different tubules within the testis. Thus, the wave of spermatogenesis resulting in development of mature sperm is a spatial cycle rather than a temporal one. The importance of growth factors and cytokines, their receptors and signal transduction pathways to gametogenesis cannot be underestimated. For example, deletion of mouse *Desert hedgehog* (*Dhh*) affects testicular development, resulting in anastomatic seminiferous tubules and an absence of adult Leydig cells. Similarly, the insulin-like growth factor (*Igf1*) null male mouse is characterized by vestigal vas deferens, prostate and seminal vesicles, caused by a steroidogenic Leydig cell defect.

Once male germ cells complete meiosis to achieve a haploid chromosomal complement, they are called spermatids. Spermatids undergo a process of cellular differentiation known as spermiogenesis, progressing from round to elongating to

elongated spermatids, culminating in the development of spermatozoa. Many male-specific genes are involved in this extensive cellular remodelling and concomitant condensation of the chromatin (for example, *Tnp1*, *Tnp2*, *Prm1*, *Prm2*, *Tleg* and *Hsp60-2*, see Fig. 2). In common with many of the Y chromosome genes that encode RNA-binding proteins and are implicated in human infertility, an absence of similar proteins in the mouse, such as STYX, disrupts spermatid development[97]. After spermiogenesis and release of the spermatozoa from the Sertoli cells into the seminiferous tubule lumen, acquisition of motility occurs during transit through the epididymis and capacitation occurs in the oviduct (fallopian tubes) of the female genital tract. Both of these processes are required for effective penetration of the zona pellucida and egg.

In the evaluation of the infertile male, a semen sample is ordered to determine sperm count, motility and morphology. Some laboratories perform sperm function tests that predict defects in sperm–zona or egg interaction, or in penetration. However, semen analysis is not a definitive test of the fertility potential of an individual unless there are no sperm in the ejaculate. This is also true in mice. For example, FSH-β mutant mice exhibit reduced sperm counts, but fertility is normal[98]. Conversely, many mouse models are infertile and demonstrate abnormal sperm function, sperm motility (for example, *ApoB*, *CatSper*, *Dnahc1*, *Hmgb2* and *Ros* knockout mice), or morphology (for example, *Tnp2*, *Tnp1* and *Sperm1* knockout mice) with no detrimental effect on sperm count. In addition, targeted deletion of the *Acr*, *Adam2*, *Adam3*, *cabinegin*, *Pe4*, *Spam1*, *Spnr*, *Trg26*, *Idf2* or *Mdhc7* genes results in normal sperm count, motility and morphology; however, sperm function is defective (Fig. 2). As a majority of unexplained cases of infertility in human males result from spermatogenic defects (Fig. 3), the homologues of the above-described mouse genes are actively being pursued for their possible roles in human infertility.

Chromosomal abnormalities are observed in 5.8% of infertile males[97] and more commonly involve sex chromosomes (4.2%), as opposed to autosomes (1.5%; Fig. 1). In addition to *SRY*, other Y chromosome genes are required for spermatogenesis. This became obvious in the XX *Sxr* male mouse and the XX *Sry* transgene positive male mouse, which are sex reversed, but display spermatogenesis blocks[51]. Similarly, a region at Yq11 that is deleted in several infertile men was termed the azoospermia factor (*AZF*) region[99]. In general, the reported incidence of deletions in this region in severe oligospermic/azoospermic men is 10–18% and varies depending on the stringency of diagnostic classification[99]. This region (now further subdivided into *AZFa*, *AZFb* and *AZFc*), contains several genes involved in spermatogenesis, including deleted in azoospermia (*DAZ*)[100,101]. In the mouse, disruption of the testis-expressed *Dazla* homologue gene on chromosome 17 abrogates gamete production. Other putative evolutionarily conserved spermatogenesis genes have been mapped to Y chromosome regions commonly deleted in infertile men (reviewed in ref. 99). Mutations in the human gene *USP9Y* (ubiquitin specific protease 9, Y chromosome or DFFRY), a homologue of the *D. melanogaster* development *Fat facets* gene[21], cause infertility[102]. Functional analysis of additional Y chromosome genes in the mouse has been complicated by the presence of multiple copies or X-chromosome homologues, as well as technical difficulties related to the low efficiency of Y chromosome homologous recombination in embryonic stem cells. However, it is expected that the recent and exciting technological breakthroughs achieved by Bishop and colleagues[103], who developed a method for successful gene targeting of the Y chromosome in embryonic stem cells, and the development of an embryonic stem cell line[104] that will facilitate germ line transmission of Y chromosome targeted genes, will rapidly translate into an enhanced understanding of the role of specific Y chromosome genes in male reproductive function.

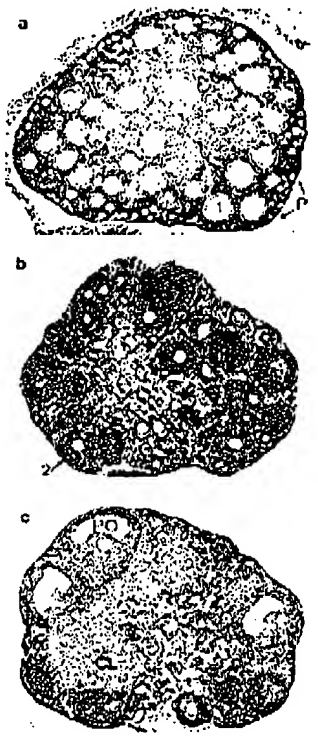

Figure 5 **Mouse knockout models to study folliculogenesis.** a, Targeted mutation of the oocyte-secreted growth factor, *Gdf9*, results in an early folliculogenesis block, resulting in an ovary with only primordial (P) and primary (1) follicles[105]. b, Absence of the endocrine hormone, FSH, results in a later block at the secondary (2) to antral follicle transition[98]. c, These knockout models contrast with wild-type ovaries that contain pre-ovulatory (PO) follicles and corpora lutea (CL). Primary- to preovulatory-stage oocytes are surrounded by a zona pellucida (magenta).

No crosstalk in females, no folliculogenesis progression

Although several proteins are involved in ovarian folliculogenesis, meiosis, and oocyte survival, oocyte–somatic cell crosstalk is especially critical for release of a fertilizable egg (Fig. 4 and ref. 105). Without the helix-loop-helix protein factor in the germline α (FIGα), pre-granulosa (somatic) cells fail to form a monolayer around individual primordial oocytes, resulting in rapid germ cell depletion from the neonatal mouse ovary and sexual infantilism[106]. Similarly, oocyte growth during folliculogenesis is regulated by signalling of

granulosa KITL to the oocyte expressed KITR[96]. KITL expression is controlled by both hormonal (FSH) and oocyte (growth differentiation factor 9 (GDF-9)) factors[107]. In the absence of the TGF-β superfamily ligand GDF-9 in mice[108] or its close oocyte-specific homologue, BMP-15, in sheep[58], an arrest in folliculogenesis at the primary follicle stage is observed (Fig. 5). FSH has no effect on the 'arrested' primary follicles of *Gdf9* knockout ovaries[108], suggesting that GDF-9 allows the granulosa cells to grow and acquire the competence to respond to FSH. Absence of GDF-9 results in elevated levels of KITL[109], which signals back to markedly increase oocyte size[108]. These findings were confirmed by studies showing that recombinant GDF-9 downregulates levels of *Kitl* mRNA[107]. In addition to oocyte factors, FSH[96] functions with IGF-1 (by stimulating cyclin D2 and oestrogen synthesis)[110,111] to regulate the growth of the follicle through the pre-ovulatory stage (Fig. 5). In pre-ovulatory follicles, LH, in conjunction with the oocyte-secreted proteins GDF-9 and BMP-15, signals to somatic cells to initiate ovulation of a healthy cumulus oocyte complex (COC). Thus, important crosstalk between somatic cells and oocytes, as well as endocrine signalling, is necessary for normal folliculogenesis and ovulation.

Post-fertilization, *Mater* (maternal antigen that embryos require) and several other genes (including *Dnmt1o*, *Pms2* and *Hsf1*) (Fig. 4), have been identified by knockout mouse studies as maternal effect (oocyte synthesized) genes that are essential for development[112]. The human homologue of *Mater* has been identified and may be a candidate gene for premature ovarian failure[113]. Similarly, several uterine proteins are required for implantation (Fig. 4 and ref. 114). Thus, these studies have pinpointed multiple putative diagnostic targets in women who present with infertility.

In women, several syndromes — including ovarian failure and infertility — are attributed to autosomal recessive mutations[88,115]. Blepharophimosis/ptosis/epicanthus inversus syndrome, the only autosomal dominant disorder associated with premature ovarian failure (POF), is caused by mutations in the forkhead transcription factor gene (*FOXL2*)[116]. Expansion of a CGG trinucleotide repeat of the Xq27.3 fragile X mental retardation gene (*FMR1*) to over 200 repeats is the most common heritable cause of mental retardation. The unstable premutation *FMR1* allele (60–199 CGG repeats) causes POF in 21% of heterozygote carriers and increased twin pregnancies[117]. Furthermore, 2% of sporadic cases and 14% of familial cases of POF are associated with the premutation allele. The pathophysiology of the premutation allele in POF is unknown, but this finding clearly represents a step forward in identifying a genetic locus for POF. To date, all other identified single gene autosomal dominant or recessive mutations with isolated infertility in humans affect steroidogenic or gonadotropin pathways, often in both sexes. However, many candidate genes await analysis in human idiopathic infertility cases.

Descent of the testis and problems with sperm transit

Testis determination and gametogenesis are necessary, but not sufficient, for male fertility, as testicular descent down the inguinal canal into the scrotum, in addition to the development of the genital tract and penis, are also critical. Mutations of the mouse genes *Insl3*, *Great* (G protein coupled receptor that affects testicular descent; a possible relaxin receptor) and *Hoxa10* (refs 118–122) result in male infertility secondary to cryptorchidism. The second phase of testicular descent requires androgens and a functional androgen receptor. In humans, cryptorchidism results from anti-Mullerian hormone (AMH) deficiency caused by obstruction of the genital tract.

Gonadal sex determines secondary duct differentiation. In females, the Müllerian duct differentiates into the oviducts, uterus and upper portion of the vagina; in males, the Wolffian duct differentiates into epididymis, vas deferens and seminal vesicles[19,20,123]. The Müllerian duct regresses in response to prenatal production of testicular AMH, and Wolffian duct development requires testosterone. Differentiation of the prostate and male external genitalia is driven by dihydrotestosterone, a product of the conversion of testosterone by 5 α reductase. Mutations in genes that affect steroidogenesis (for example, P450 aromatase (*CYP19*) and 5-α reductase) and steroid signalling pathways (for example, oestrogen receptor α (*ERα*) and androgen receptor) have deleterious effects on genital tract development and function in the male. Thus, it follows that pseudohermaphroditism occurs as a result of defects in genes involved in gonad formation (for example, *SF1* and *WT1*). Mutations of the AMH or AMH receptor genes result in persistence of Müllerian duct syndrome (PMDS), resulting in obstructive azoospermia and fertility defects in men, male dogs and mice (Fig. 1 and ref. 123).

One to two per cent of infertile men present with obstructive azoospermia caused by congenital bilateral absence of the vas deferens (CBAVD), as a result of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene[124]. These CBAVD patients successfully father offspring because microsurgical epididymal sperm aspiration yields 'normal' sperm for *in vitro* fertilization (IVF). Male fertility also may be compromised by epididymal, ejaculatory or erectile dysfunction, as well as by other congenital anomalies.

New technologies and perspectives

Genome, gene and cDNA sequences are being deposited into public databases (for example, the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) or the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk)) with amazing speed. Furthermore, programs to search these databases, such as BLAST (http://www.ncbi.nlm.nih.gov/blast) and the Unigene database at NCBI, are helping scientists to use this wealth of information. In particular, sequences unique to mammalian germ cells have been identified using an *in silico* subtraction (electronic database) approach[125]. For example, *GASZ* (germ-cell-specific and ankyrin repeat, sterile α motif and basic leucine-zipper-containing protein) was identified as a

novel evolutionarily-conserved germ cell-expressed gene lying adjacent to the *CFTR* gene in human, chimpanzee, baboon, cow, rat and mouse[1-26]. Functional expression and sequence data is also being collated into collections, such as the Ovarian Kaleidoscope database (http://ovary.stanford.edu), the Male Reproductive Genetics database (http://mrg.genetics.washington.edu/home.html) and the GermOnline database (http://germonline.igh.cnrs.fr). With the use of microarrays for expression analysis of reproductive tissues[127], these 'bits' of data will increase exponentially. Therefore, there is an urgent need for bioinformatics advances to facilitate compiling and sorting through this wealth of *in silico* information for future applications in the clinic.

Technological procedures and advances in the clinic are also wrought with some controversies and dilemmas. Of particular importance, the application of assisted reproductive technologies (ART) for severe male and female factor infertility serves to not only overcome sterility, but bypasses natural barriers to the inheritance of defective genes. This results in considerable concern that genetic defects will be transmitted to the next generation. Among these controversial treatments are ICSI (intracytoplasmic sperm injection), cytoplasmic (ooplasmic) transfer, round spermatid nuclear injection (ROSNI or ROSI) and reproductive cloning (described in more detail by Schatten in this issue[128]).

These technologies have prompted significant debates concerning their morality and safety. However, even a United States government moratorium on human cloning has not deterred renegade scientists overseas from actively engaging in this research, which could result in a potentially disastrous outcome.

Conclusions

To date, diagnosis of infertility in the clinic has been hindered by our relatively poor understanding of the underlying molecular mechanisms. Although investigators have attempted to translate findings in animal models to humans by searching for gene mutations/deletions in idiopathic infertility patients, in general, these investigations have not been fruitful. Given the large number of candidate evolutionarily conserved 'fertility' genes yet to be discovered or identified from mutant mouse studies, and the overall complexity of the reproductive system in general, proper diagnosis and treatment of these patients will await the development of more sophisticated and rapid technologies. Finally, if mutation of a gene in mice or humans results in infertility, the protein product of that gene may be a future target for novel contraceptives that are designed to transiently or permanently cause infertility.

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Online Table: Mouse mutations causing reproductive defects. Only single mutant defects are described. Fertility defects of unknown gene origin are not described. M, male; F, female; Hetero, heterozygote phenotype

Mutant gene	Sex Affected	Reproductive Phenotype	Fertility Status	References
Acrosin (*Acr*)	Males	Sperm are capable of binding and penetrating the zona pellucida	Delayed fertility	1
Activin receptor-type IIA (*Acvr2*)	Both	Antral follicle block in females; small testes, delayed fertility in males	Infertility (F) Subfertility (M)	2
Activin/inhibin βB subunit (*Inhbb*)	Female	Delivery and nursing defects	Subfertility	3
Acyl-CoA synthetase 4 (ACS4; *Facl4*)	Female (Hetero)	Enlarged uteri with prostaglandin accumulation	Subfertility	4
Adamts1 (a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 1)	Female	Cystic formations in uteri; defects in preovulatory follicle development	Subfertility	5
Adamts2 (procollagen N-proteinase)	Male	Defects in spermatogenesis; marked decrease in sperm within testes tubules	Infertility	6
ADP-ribosylation factor-like 4 (*Arl4*)	Male	Significantly reduced testicular weights and sperm counts	Normal fertility	7
Alpha 1 microglobulin/bikunin (*Amhp*; Urinary trypsin inhibitor)	Female	Defects in ovulation and cumulus-oocytes complex (COC) integrity	Subfertility	8
Angiotensin-converting enzyme (*Ace*)	Male	Compromised ability of sperm to fertilize ova	Subfertility	9
Androgen receptor (*Ar*; *tfm* or Testicular feminization)	Male	Feminized external genitalia; hypogonadal; cryptorchidism with a block in spermatogenesis	Infertility	10
Anti-Müllerian hormone (*Amh*)	Both	Uteri development in males causes obstruction and secondary infertility; females exhibit early delpetion of primordial follicles	Secondary Infertility	11,12
AMH receptor (*Amhr2*)	Male	Uteri development in males causes obstruction and secondary infertility	Secondary Infertility	13
Apaf1 (Apoptotic protease activating factor 1)	Male	Spermatogonial degeneration	Variable lethality; Infertility	14
Apolipoprotein B (*Apob*)	Male (Hetero)	Decreased sperm count, motility, survival time, and ability to fertilize ova	Infertility	15
Aryl-hydrocarbon receptor (*Ahr*)	Female	Early development of primordial follicles; decreased numbers of antral follicles	Subfertility	16,17
Ataxia telangiectasia (*Atm*)	Both	Germ cells degenerate; disruptions evident in meiosis I	Infertility	18,19
ATP-binding cassette transporter 1 (*Abca1*)	Female	Placental malformations leading to impaired embryo growth, embryo loss and neonatal death	Subfertility	20

Basigin (*Bsg*)	Both	Defects in fertilization and implantation (F); block in spermatogenesis at metaphase I (M)	Partial lethality; Infertility	21,22
Bax (Bcl2-associated X protein)	Both	Premeiotic arrest of spermatogenesis; increased oocytes and primordial follicles postnatally	Infertility (M)	23,24
Bcl2 (B-cell leukemia/ lymphoma 2)	Female	Fewer oocytes/primordial follicles in the post-natal ovary	Subfertility	25
Bcl6	Male	Apoptosis in metaphase I spermatocytes	Subfertility	26
Bcl X (*Bcl2l*) hypomorph	Both	PGCs are lost by E15.5	Infertility	27
Bclw (Bcl2l2, *Bcl2-like 2*)	Both	Late meiotic arrest with loss of germ cells (M) and reduced PGC survival (F)	Infertility (M) Subfertility (F)	28
Bone morphogenetic protein 4 (*Bmp4*)	Both	Absent primordial germ cell (PGC) population; defect in PGC development	Lethal	29
Bmp8a	Male	Degeneration of germ cells and epididymis	Progressive Infertility	30
Bmp8b	Both	Reduced or absent PGCs (developmental defect); Postnatal male germ cell proliferation/differentiation defect and spermatocyte apoptosis	Subfertility/ Infertility	31
Bmp15	Female	Defects in cumulus-oocyte complex (COC) formation and ovulation	Subfertility	32
BMP receptor, type IB (*Bmpr1b*)	Female	Defects in estrous cyclicity, cumulus expansion, and endometrial gland development	Subfertility	33
Calmegin (*Clgn*)	Male	Defect in sperm-zona pellucida binding	Infertility	34
Camk4 (calcium/calmodulin-dependent protein kinase IV)	Male	Impaired chromatin packaging during spermiogenesis	Infertility	35
cAMP-responsive element modulator (*Crem*)	Male	Defective spermiogenesis with aberrant post-meiotic gene expression	Infertility	36,37
cAMP-specific phosphodiesterase type 4 (*Pde4d*)	Female	Diminished sensitivity of the granulosa cells to gonadotropins	Subfertility	38
Casein kinase IIα 1 (*Csnk2a2*)	Male	Globozoospermia (no acrosomal cap)	Infertility	39
Caspase-2 (*Casp2*)	Female	Decreased apoptosis of female germ cells	Increased Fertility	40
CatSper (putative sperm cation channel)	Male	Defects in motility and fertilization	Infertility	41
CD9 antigen (*Cd9*)	Female	Sperm-egg binding defect	Subfertility	42
Cell division cycle 25 homolog B (*Cdc25b*) (Cdc25b phosphatase)	Female	Oocytes are arrested in meiotic prophase, with defects in maturation promoting factor activity	Infertility	43
Centromere protein B (*Cenpb*)	Both	Males are hypogonadal and have low sperm counts; females have strain-dependent uterine epithelium defects	Subfertility (F)	44,45

C/EPBβ (CCAAT/enhancer-binding protein β)	Female	Reduced ovulation and block in CL differentiation	Infertility	46
Claudin 11 (*Cldn11*; Osp-11)	Male	No tight junctions between Sertoli cells	Infertility	47
Colony stimulating factor (macrophage) (*Csf1*)	Both	Males have reduced testosterone; females have implantation and lactation defects	Subfertility	48
Colony stimulating factor (granulocyte-macrophage) (*Csf2*)	Both	Mean litter size decrease with disproportionate loss of males pups (F); maternal effects most pronounced in intercrosses with knockout males	Intercrossing Subfertility	49
Connexin 37 (*Gja4*; Cx37)	Female	Defects in late folliculogenesis and oocyte meiosis	Infertility	50
Connexin 43 (*Gja1*; Cx43)	Both	Small ovaries and testes; decreased numbers of germ cells from E11.5	Neonatal lethality	51
Cpeb (cytoplasmic polyadenylation element binding protein)	Both	Disrupted germ cell differentiation and meiosis I synaptonemal complex formation	Infertility	52
Cut-like 1(*Cutl1*; CDP/Cux) truncation mutant	Male	Severely reduced male fertility	Subfertility	53
Cyclin A1 (*Ccna1*)	Male	Block in spermatogenesis before the first meiotic division	Infertility	54
Cyclin D2 (*Ccnd2*)	Both	Failure of granulosa cell proliferation (F); males fertile with decreased testis size	Infertility (F)	55
Cyclin dependent kinase 4 (*Cdk4*)	Female	Defects in the hypothalamic-pituitary-gonadal axis	Infertility	56
Cyclooxygenase 2 (*Ptgs2*)	Female	Defects in ovulation and implantation	Most Infertile	57,58
Cyp11a (Cytochrome P450, 11a, cholesterol side chain cleavage)	Both	Males feminized with female external genitalia, underdeveloped sex organs; gonads degenerate	Lethal	59
Cyp19 (Cytochrome P450, 19, aromatase)	Both	Early spermatogonial arrest, Leydig cell hyperplasia, and defects in sexual behavior (M); folliculogenesis block and ovulation defects (F)	Progressive Infertility (M); Infertility (F)	60-62
Cyp40 (P450 25-hydroxyvitamin D-1α–hydroxylase)	Female	Uterine hypoplasia and absence of CL	Infertility	63
Cyritestin (*Adam3*)	Male	Altered sperm protein expression and adhesion defects during fertilization	Infertility	64,65
Dax1 (*Nr0b1*)	Male	Progressive degeneration of the germinal epithelium	Infertility	66
Dazl (Deleted in azoospermia-like)	Both	Reduced germ cells; differentiation failure and degeneration of germ cells	Infertility	67
Desert hedgehog (*Dhh*)	Male	Complete absence of mature sperm; defects in Sertoli-to-Leydig cell	Infertility	68,69

		signaling		
Dmc1h (Disrupted meiotic cDNA 1 homolog)	Both	Defects in chromosome synapsis in meiosis; female germ cells degenerate during embryogenesis	Infertility	70,71
Dnmt1o (DNA methyltransferase)	Female	Embryos of knockout females die during gestation due to imprinting defects; maternal effect gene	Subfertility	72
DNA polymerase λ	Male	Immotile spermatozoa	Lethality; Infertility	73
Doublesex and mab-3 related transcription factor 1 (*Dmrt1*)	Male	Defects in post-natal testes differentiation; disorganized seminiferous tubules and absence of germ cells	Infertility	74
Dynein heavy chain 7 (*Dnahc1*)	Male	Defects in sperm flagellar motility	Infertility	75
Early growth response 1 (*Egr1*; NGFI-A) targeted *lacZ* insertion	Both	Lack of LH (M); downregulation of LHR, not remedied with gonadotropin treatment (F)	Infertility	76
Early growth response 1 (*Egr1*) targeted *neo* insertion	Female	LH insufficiency; loss of estrous cyclicity, no CL; rescued by treatment with gonadotropins	Infertility	77
Early growth response 4 (*Egr4*)	Male	Germ cells undergo apoptosis during pachytene stage	Infertility	78
ELKL motif kinase (*Emk*)	Both	β-gal gene trap insertion creates a null allele; homozygotes intercrossed are not fertile	Intercrossing Infertility	79
Empty spiracles homolog 2 (*Emx2*)	Both	Defective development of gonads and urogenital tracts	Lethal	80
Estrogen receptor α (ERα)(*Esr1*)	Both	Females have hemorrhagic ovarian cysts and uterine defects, decreased lordosis response; males develop disruptions of the seminiferous epithelium due to abnormal epididymal function, no ejaculations	Infertility	81-84
Estrogen receptor β (ERβ)(*Esr2*)	Both	Females are subfertile; males are fertile, but develop prostate hyperplasia	Subfertility	85
Fanconi anemia complementation group A (*Fanca*)	Both	Hypogonadism, reduced fertility, more dramatic and progressive in females	Subfertility	86
Fanconi anemia complementation group C (*Fancc*)	Both	Hypogonadism, compromised gametogenesis	Subfertility	87,88
Fanconi anemia complementation group G (*Fancg*)	Both	Hypogonadism, compromised gametogenesis	Subfertility	89
Fertilin β (*Adam2*)	Male	Altered sperm protein expression and adhesion defects during fertilization	Infertility	65,90
Fibroblast growth factor 9 (*Fgf9*)	Male	XY male-to-female sex reversal; phenotype ranges from testicular hypoplasia to complete sex reversal	Lethal	91
Figla or FIGα (Factor	Female	No primordial follicles develop at birth	Infertility	92

in the germline α)		and oocytes die		
Fragile-X mental retardation syndrome 1 homolog (*Fmr1*)	Male	Macroorchidism		93
FSH hormone β-subunit (*Fshb*)	Both	Female pre-antral block in folliculogenesis; males decreased testis size	Infertility (F)	94
FSH receptor (*Fshr*)	Both	Female pre-antral block in folliculogenesis; males decreased testis size	Infertility (F)	95
Fus1 (translocated in liposarcoma; TLS)	Male	Defects in spermatocyte chromosome pairing	Infertility	96
β1,4-Galactosyltransferase	Both	Male infertility; defects in sperm-egg interaction; females exhibit defects in delivery and lactation	Variable lethality	97,98
γ-Glutamyl transpeptidase (*Ggtp*)	Both	Both males and females are hypogonadal and infertile; phenotype corrected by feeding mice N-acetylcysteine	Infertility	99,100
Gdi1 (guanosine diphosphate dissociation inhibitor 1; Rho GDIα)	Both	Impaired spermatogenesis, vaculolar degeneration in males; post-implantation pregnancy defects in females	Infertility	101
Glial cell line-derived neurotrophic factor (*Gdnf*)	Male (Hetero)	Depletion of stem cell reserves; spermatogonia differentiate	Fertile	102
Glycoprotein hormone α-subunit (*Cga*)	Both	Hypogonadal due to FSH and LH deficiency	Infertility	103
Gpr106 (G protein-coupled receptor 106)	Male	*Crsp* males homozygous for transgene integration exhibit a high intraabdominal position of the testes, complete sterility	Infertility	104
Growth differentiation factor-7 (*Gdf7*)	Male	Defects in seminal vesicle development	Infertility	105
Growth differentiation factor-9 (*Gdf9*)	Female	Folliculogenesis arrest at the one-layer follicle stage	Infertility	106,107
Growth hormone receptor (*Ghr*)	Female	Delayed puberty and prolonged pregnancy		108
H19	Female (Hetero)	Loss of maternal allele in developing embryos causes somatic overgrowth due to loss of IGF2 imprinting		109
Heat shock protein 70-2 (*Hsp70-2*)	Male	Meiosis defects and germ cell apoptosis	Infertility	110
Heatshock transcription factor 1 (*Hsf1*)	Female	Maternal effect gene; pre- and post-implantation defects	Infertility	111,112
Hepatocyte nuclear factor (HNF-1α)(transcription factor 1; *Tcf1*)	Both	Infantile uterus; normal ovarian histology (F); vestigial vas deferens, seminal vesicles and prostate, impaired spermatogenesis, no mating behavior	Infertility	113

		(M)		
High mobility group box 2 (*Hmgb2*)	Male	Sertoli and germ cell degeneration and immotile spermatozoa	Subfertility	114
Histone H2A family, member X (*H2afx*)	Male	Pachytene stage arrest in spermatogenesis; defects in chromosome segregation and MLH1 foci formation	Infertile	115
Histone 3.3A gene (*H3f3a*) insertional mutation	Male	β-gal gene trap insertion creates a hypomorphic allele; homozygous males have reduced copulatory activity and fewer matings result in pregnancy	Subfertility	116
Homeobox A10 (*Hoxa10*)	Both	Variable infertility; males have cryptorchidism and females have frequent embryo loss prior to implantation	Progressive infertility (M); subfertility (F)	117
Homeobox A11 (*Hoxa11*)	Both	Females have uterine defects; males have malformed vas deferens and undescended testes	Infertility	118
Hrb (HIV-1 Rev binding protein) (RAB/Rip)	Male	Round-headed spermatozoa lack an acrosome (globozoospermia)	Infertility	119
Inhibin α (*Inha*)	Both	Granulosa/Sertoli tumors, gonadotropin hormone-dependent	Infertility (F) Secondary infertility (M)	120,121
Inositol polyphosphate-5-phosphatase (*Inpp5b*)	Male	Sperm have reduced motility and reduced ability to fertilize eggs; defects in fertilin β processing	Infertility	122
Insulin-like growth factor 1 (*Igf1*)	Both	Hypogonadal and infertile; disrupted spermatogenesis and vestigial ductal system, defects in mating behavior (M); impaired antral follicle formation (F)	Infertility	123
Insulin-like growth factor 2 receptor (*Igf2r*); T-associated maternal effect (*Tme*) mutation	Female (Hetero)	Mutation of maternal allele in pups causes developmental defects and embryonic/perinatal death	Lethality; maternal effect	124
Insulin-like hormone 3 (*Insl3*)	Both	Bilateral cryptorchidism results in abnormal spermatogenesis in males; female subfertility associated with irregular estrous cycles	Subfertility	125
Insulin receptor substrate 2 (*Irs2*)	Female	Small, anovulatory ovaries with reduced numbers of follicles	Infertility	126
Interleukin 11 (*Il11*)	Female	Compromised implantation and decidualization	Infertility	127
JunD (*Jund1*)	Male	Anomalous hormone levels and sperm structural defects	Infertility	128
Kit ligand (*Kitl*)	Both	*steel* defect mutation causes defect in PGC migration/survival; *panda*	Infertility	129,130

		mutation causes blocks in folliculogenesis in females		
Kit receptor (*Kit*)	Both	*White spotting* null mutation causes PGC defects	Infertility	131
Leptin (*Lep; ob/ob*) mutant	Both	Obese and infertile with hypogonadotropic hypogonadism	Infertility	132,133
Leptin receptor (*Lepr; db/db*) mutant	Both	Obese and infertile with hypogonadotropic hypogonadism	Infertility	134
Leukemia inhibitory factor (*Lif*)	Female	Failed implantation	Infertility	135
Limk2 (LIM motif-containing protein kinase 2)	Male	Degeneration of spermatogenic cells in the seminiferous tubules; increased apoptosis		136
Lipase, hormone sensitive (HSL) (*Lipe*)	Male	Multiple abnormalities in spermatogenesis	Infertility	137,138
Luteinizing Hormone Receptor (*Lhcgr*)	Both	Underdeveloped sex organs and infertility in both males and females; spermatogenesis arrested at round spermatid stage; preantral folliculogenesis block	Infertility	139,140
Man2a2 (α-mannosidase IIx)	Male	Defect in adherence of spermatogenic cells to Sertoli cells; germ cells prematurely released from the testis	Mostly infertile	141
Mater (maternal antigen that embryos require)	Female	Development beyond the two-cell stage is blocked; Maternal effect gene	Infertility	142
Mlh1 (MutL homologue 1)	Both	Meiotoc arrest and genomic instability	Infertility	143,144
Mos (Moloney sarcoma oncogene)	Female	Parthenogenetic activation, cysts and teratomas	Subfertility	145,146
Msh4 (MutS homologue 4)	Both	Prophase I meiotic defects apparent at the zygotene/pachytene stage; germ cells lost within a few days post-partum	Infertility	147
Msh5 (MutS homologue 5)	Both	Zygotene/pachytene meiotic defects with aberrant chromosome synapsis and apoptosis	Infertility	148,149
Microtubule-associated protein (*Mtap7*)(E-MAP-115) insertional mutation	Male	Abnormal microtubules in germ cells and Sertoli cells	Infertility	150
More (microrchidia) insertional mutation	Male	Early arrest in meiosis and germ cell apoptosis	Infertility	151
Mybl1 (A-myb) myeloblastosis oncogene-like 1	Male	Germ cell meiotic arrest at the pachytene stage	Infertility	152
$Na^{(+)}$-$K^{(+)}$-$2Cl^{(-)}$ cotransporter (NKCC1) solute carrier family 12, member 2 (*Slc12a2*)	Male	Low spermatid counts and compromised sperm transport	Infertility	153
Neuronal Helix-Loop-Helix 2 (*Nhlh2*)	Both	Males are infertile and hypogonadal; females are fertile when reared with	Infertility	154

		males		
Neuronal insulin receptor (NIR)	Both	Hypothalamic hypogonadism; impaired spermatogenesis and follicle maturation	Infertility	155
Nitric oxide synthase 3, endothelial cell (*Nos3*; eNos)	Female	Compromised ovulation, delayed meiotic progression from metaphase I	Subfertility	156
Nuclear receptor co-activator (*Ncoa1*); steroid receptor coactivator-1 (SRC1)	Both	Decreased responsiveness to steroid hormones in uterus, mammary glands (F), testes and prostate (M)	Fertile	157
Nuclear receptor co-repressor RIP40 (*Nrip1*)	Female	Ovulation defect; ovaries accumulate luteinized, unruptured follicles	Infertility	158
Nuclear receptor subfamily 5, group A, member 1 (*Nr5a1*); Steroidogenic factor-1 (SF-1)	Both	Gonadal agenesis in both sexes	Lethal	159
Otx1(orthodenticle homolog 1)	Both	Prepubescent dwarfism and hypogonadism; progressive recovery of follicular development and sperm development and fertility	Delayed fertility	160
Ovo	Male	Reduced fertility and underdeveloped genitalia	Subfertility	161
P2X1 receptor (*P2rx1*)	Male	Oligospermia and defective vas deferens contraction	Infertility	162
$p18^{Ink4c}$ (*Cdkn2c*)	Male	Leydig cell hyperplasia and reduced testosterone production	Fertile	163
$p19^{Ink4d}$ (*Cdkn2d*)	Male	Testicular atrophy and germ cell apoptosis	Fertile	164
$p27^{Kip1}$ (*Cdkn1b*)	Both	CL differentiation failure and granulosa cell hyperplasia (F); males fertile with testicular hyperplasia	Infertility (F)	165,166
$p57^{Kip2}$ (*Cdkn1c*)	Both	Surviving mice show sexual immaturity	Mostly lethal	167
PAC_1; adenylate cyclase activating polypeptide 1 receptor 1 (*Adcyap1r1*)	Female	Prolonged and irregular diestrous phase	Subfertility	168
PC4 (testicular germ cell protease) (*Pcsk4*)	Male	Sperm have impaired fertilization ability	Infertility	169
Pentraxin 3 (*Ptx3*)	Female	Defects in cumulus-oocyte complex (COC) integrity and ovulation	Subfertility	170
Phosphatidylinositol 3'-kinase (*Pi3k*)	Male	Defects in proliferation and increased apoptosis of spermatogonia	Infertility	171
Phosphatidylinositol glycan, class A (*Piga*)	Chimeric Male	Abnormal testes, epididymis and seminal vesicles	Variable Infertility; no allele transmission	172
Pit1 (pituitary specific transcription factor 1)	Both	*Snell* dwarf mice have multiple anterior pituitary hormone deficiencies and	Infertility	173

		hypogonadism		
Polyomavirus enhancer activator 3 (*Pea3*)	Male	Normal mating behavior, but males do not set plugs or release sperm	Infertility	174
Postmeiotic segregation increased 2 (*Pms2*)	Both	Abnormal chromosome synapsis in meiosis (M); female knockout zygotes have microsatellite instability in both maternal and paternal genomes; Maternal effect gene	Infertility (M)	175,176
Progesterone receptor (*Pgr*)	Female	Defects in ovulation, implantation, sexual behavior, and mammary gland development	Infertility	177
Prolactin (*Prl*)	Female	Females are infertile with irregular estrus cycles	Infertility	178
Prolactin receptor (*Prlr*)	Both	Compromised ovulation, fertilization and preimplantation development in knockouts (F); defects in maternal behavior in knockouts and heterozygotes (F); variable infertility and subfertility in males	Infertility (F); Subfertility (M)	179,180
Prop1 (paired like homeodomain factor 1; prophet of *Pit1*)	Both	*Ames* dwarf mice have multiple anterior pituitary hormone deficiencies and hypogonadism	Infertility	181
Prostaglandin E2 EP2 receptor (*Ptger2*)	Female	Decreased fertilization and defects in cumulus expansion	Subfertility	182-184
Prostaglandin F receptor (*Ptgfr*)	Female	Females do not undergo parturition; failed luteolysis	Infertility	185
Protamine 1 (*Prm1*)	Chimeric Male	Protamine haploinsufficiency; abnormal spermatogenesis	Infertility	186
Protamine 2 (*Prm2*)	Chimeric Male	Protamine haploinsufficiency; abnormal spermatogenesis	Infertility	186
Protease inhibitor protease nexin-1 (PN-1) knockout (*Serpine2*)	Male	Abnormal seminal vesicle morphology and altered semen protein composition	Subfertility	187
Protein kinase A, catalytic subunit α (*Prkaca*)	Male	Most mice die; few viable mice have sperm motility defects	Mostly lethal	188
Protein phosphatase 1 catalytic subunit γ (*Ppp1cc*)	Male	Defects in spermiogenesis	Infertility	189
Protein phosphatase 1 regulatory subunit 1B (*Ppp1r1b*)(DARPP-32)	Female	Knockouts exhibited defects in progesterone facilitated sexual receptivity	Not reported	190
Puromycin-sensitive aminopeptidase (*Psa*)	Female	Lack of CL formation and prolactin production cause early pregnancy loss	Infertility	191
Retinoic Acid Receptor alpha (*Rara*)	Male	Complete arrest and degeneration or germ cell depletion	Infertility	192
Retinoic acid receptor γ (*Rarg*)	Male	Squamous metaplasia of the seminal vesicles and prostate	Infertility	193
Retinoid X receptors (*Rxrb*)	Male	Germ cell maturation defects and tubular degeneration	Infertility	194
Ros1 (c-ros	Male	Sperm motility defects	Infertility	195,196

protoncogene)				
Scavenger receptor, class B1 (*Srb1*)	Female	Defects in oocyte maturation and early embryo development due to abnormal lipoprotein metabolism	Infertility	197-199
Serpina5 (Serine proteinase inhibitor A 5; Protein C inhibitor)	Male	Sertoli cell destruction	Infertility	200
SH2-B	Both	Males have small testes and reduced sperm count; females have small, anovulatory ovaries with reduced numbers of developing follicles	Subfertility (M) Infertility (F)	201
Smad1 (MAD homolog 1; *Madh1*)	Both	Developing embryos lose PGCs	Lethal	202
Smad5 (MAD homolog 5; *Madh5*)	Both	Developing embryos lose PGCs	Lethal	203
Sp4 trans-acting transcription factor (*Sp4*)	Male	Defects in reproductive behavior	Infertility	204
Spam1 (sperm adhesion molecule) mutations	Female	Sperm defects in hyaluronic-acid binding	Subfertility	205
Sperm-1	Male	Defect in haploid sperm function	Subfertility	206
Sperm mitochondrion-associated cysteine-rich protein (SMCP)	Male	Defects in sperm motility and migration into the oviduct; defects in fertilization	Subfertility and Infertility	207
Spermatid perinuclear RNA-binding protein (*Spnr*) insertional mutation	Male	Defects in seminiferous epithelium and spermatogenesis	Subfertility	208
SPO11 homolog (*Spo11*)	Both	Defects in meiosis; oocytes lost soon after birth	Infertility	209,210
Steroid 5α-reductase type 1 (*Srd5a1*)	Female	Defects in parturition	Infertility	211,212
Steroidogenic acute regulatory protein (*Star*)	Both	Males have female external genitalia; both sexes die of adrenocortical insufficiency	Lethal	213
Styx (phosphoserine/ threonine/tyrosine interaction protein)	Male	Defects in round and elongating spermatid development	Infertility	214
Superoxide dismutase 1 (*Sod1*)	Female	Folliculogenesis defect; failure to maintain pregnancy	Subfertility	215,216
Sycp3 (synaptonemal complex protein 3)	Both	Defects in chromosome synapsis during meiosis; germ cell apoptosis in males; embryonic loss in females due to aneuploidy	Infertility (M) Subfertility (F)	217,218
Taf4b (TAF4B RNA polymerase II, TATA box binding protein-associated factor; TAFII105)	Female	Defects in follicular development, oocyte maturation/fertilization	Infertility	219
TATA-binding protein-like protein (*Tlp*;	Male	Post-meiotic spermiogenesis block (defective acrosome formation in early	Infertility	220,221

TRF2)		stage spermatids)		
Telomerase reverse transcriptase (*Tert*)	Both	Progressive infertility in both sexes; females have few oocytes and uterine abnormalities	Progressive Infertility	222
Theg (*kisimo*) (Transgene integration)	Male	Abnormal elongated spermatids; asthenospermia	Infertility	223
Thyroid stimulating hormone β (*Tshb*; *hyt/hyt*) mutant	Female	Hypothyroid; females show continuous dioestrus, and poor response to gonadotropin-induced superovulation	Infertility	224
Tial1 (cytotoxic granule-associated RNA binding protein-like 1)	Both	PGCs lost by E13.5	Infertility	225
Tnp1 (transition protein 1)	Male	Abnormal chromosome condensation, sperm motility	Subfertility	226
Tnp2 (transition protein 2)	Male	Abnormal chromosome condensation	Subfertility	227
Tumor necrosis factor type I receptor (*Tnfrsf1a*)	Female	Enhanced prepubertal response to gonadotropins; early ovarian senescence	Subfertility	228
Ube2b (E2B ubiquitin-conjugating enzyme; HR6B)	Male	Alterations in sperm chromatin structure, an incomplete meiotic arrest, abnormal sperm morphology	Infertility	229
Ubiquitin-like DNA repair gene HR23B (*Rad23b*)	Male	Most knockouts die during development or shortly after birth; surviving mice have multiple abnormalities and male sterility	Variable lethality; Infertility	230
Ubiquitin protein ligase E3A (*Ube3a*; E6-AP ubiquitin protein ligase)	Both	Testicular hypoplasia, defects in spermatogenesis and prostate gland development (M); ovarian hypoplasia, defects in ovulation and uterine development (F)	Subfertility	231
Ubiquitin protein ligase seven in absentia 1A (*Siah1a*)	Male	Block in spermatogenesis and germ cell apoptosis; failure to complete transition to telophase of meiosis I	Partially lethal; Infertility	232
VASA homolog (*Ddx4*; DEAD box polypeptide 4)	Male	Defective proliferation/differentiation of PGCs	Infertility	233
Vitamin D receptor (*Vdr*) knockout	Both	Defects in estrogen biosynthesis in males and females; elevated serum gonadotropins	Infertility	234,235
Voltage-dependent Anion Channel Type 3 (*Vdac3*)	Male	Immotile sperm; axonemal defects with sperm maturation	Infertility	236
Wilms tumor homolog (*Wt1*)	Both	Gonadal agenesis	Lethal	237
Wip1 (p53-induced phosphatase)	Male	Runting and testicular atrophy	Subfertility	238
Wingless-related MMTV integration site 4 (*Wnt4*)	Female	Ovaries depleted of oocytes; Müllerian ducts do not form	Infertility	239

Wnt7a	Both	Females show abnormal development of oviducts and uterus; males do not have Müllerian duct regression	Infertility	240
Zfx (Zinc finger protein X-linked)	Both	Reduced germ cell numbers; males have reduced sperm, but are fertile; females subfertile	Subfertility (F)	241
Zona pellucida protein 1 (*Zp1*)	Female	Defects in fertilization	Subfertility	242
Zp2	Female	Fragile oocytes with defects in developmental competence	Infertility	243
Zp3	Female	Fragile oocytes	Infertility	244,245

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